THE FUNGOUS ROOT-TUBERCLES OF CEANOTHUS AMERICANUS, ELAEAGNUS ARGENTEA, AND MYRICA CERIFERA.*

BY E. G. ARZBERGER.

INTRODUCTION.

Although considerable work has been done by several investigators on the peculiar root-tubercles found on the alder and some other plants, no satisfactory account of their nature, origin, and function has yet been fully set forth. Especially the question of their relation to the so-called mycorrhiza remains to some extent unsolved. The following studies were undertaken with the hope of getting fuller information regarding the gross structure, physiology and cytology of the forms which occur on the roots of *Ceanothus, Elaeagnus* and *Myrica.*

HISTORICAL.

A brief resumé of the leading views concerning the root tubercles, from a historical standpoint, is interesting from the fact that, for more than three decades, these structures on the alder were described by various investigators, none of whom proceeded far enough to determine the true nature of the tubercles and the fungus which causes them.

Meyen, (23) in 1829, gives the first description of the tubercles on the alder and considers them as "pseudomorphosed roots," in the ends of which there is a parasitic growth comparable to that of *Lathraea, Rafflesia* and *Balanophora,* though of a more primitive nature and in many respects resembling growths of a parasitic origin, found in an animal body. He claimed that the tubercles are formed when the alders grow near flowing water and in shady places.

---

* A thesis submitted to the Faculty of Washington University in candidacy for the degree of Master of Arts, June, 1910.

Schacht (32-37) in each of his six productions on plant anatomy and physiology, refers to the tubercles found on the alder. He sets forth their morphology with description and illustrations, and at first states that they are only normal growths, but in later articles he considers them as abnormal growths of the roots; however, no fact or theory regarding an organism which may cause them is presented.

Döbner (8), in his Lehrbuch, mentions the tubercles formed on the roots of alder seedlings and regards them as roots altered by much branching and thickening. No reference is made to parasitism.

Jäger (18) considers the tubercles as insect galls similar to those found on the twigs of the willow and the pine.

Rossmässler (31), in his description of the roots of the alder, mentions the rusty-brown cluster-like outgrowth found on large and small alder plants.

In 1866 Woronin (56) first described and presented figures of the fungus that produces the tubercles on various species of the alder. He considered it closely related to the genus *Schinzia* of Nägeli, and proposed for it the name *Schinzia alni.* Later he made additional investigation on young tubercles and stated that two organisms, one resembling *Plasmodiophora brassicae* and another a filamentous type, may be present in the cells. In the latter view he was supported by Möller (24), who called the fungus *Plasmodiophora alni.*

Ratzeburg (30) refers to the abundance of tubercles on the alders and gives a full description accompanied with plate figures, some of which were borrowed from Woronin. He predicts that an organism belonging to the lower cryptogams will be found in the cells.

Möller (24) supported the views of Woronin regarding the nature of the fungus and proposed the name *Plasmodiophora alni.* Five years later (25, 26) he reinvestigated fresh material and retracted his earlier views in favor of those set forth by Brunchorst (6), who, by a thorough investigation, arrived at the true nature of the fungus in a study of the tubercles of *Alnus* and *Elaeagnus.* With a more modern technique he was able to determine the mycelial nature of

the fungus and its relation to the sporange-like structures which Woronin and Möller considered as spores. Brunchorst found that the content of the sporange segments into several parts which he considers as spores. In the longitudinal section he points out three distinct stages through which the fungus passes in its development. He regarded the fungus as distinct from *Schinza* and *Plasmodiophora* and considered it a new genus, naming it *Frankia subtilis,* though admitting that the fungus varies morphologically in different host plants.

Frank (9, 10), in his first contribution, sets forth some peculiar views in regard to the fungus, considering the tubercles as normal growths for the transitional storage of proteid material. He mentions the vesicles and the various changes which they undergo. In a later publication he revises his former views and in many respects corroborates the conclusions of Brunchorst. He did not succeed in obtaining growths from cultures but describes very clearly the intercellular infection, the effect of the fungus on the cell and its nucleus, and the symbiotic relationship of the host cell and the fungus. However, he did not believe with Brunchorst that the vesicles are fruiting bodies with the dividing contents comparable to spores. As the most appropriate name for the fungus Frank proposed "Ernährungsphysiologische Mycorrhiza."

Atkinson (1) is the first who investigated the root-tubercles of *Ceanothus americanus,* which were discovered in 1890 by Dr. W. J. Beal in Michigan. On examining fresh material he found the organism producing structures closely allied to that which is found in the tubercles of *Alnus, Elaeagnus,* and *Myrica,* hitherto so well known in Europe. He presented in his paper an accurate description of the individual tubercles as regards color, shape, size, method of branching, the formation of large clusters, the various layers of tissue and the cell, finding that the internal structure of the tubercle varies but little in relation to the various tissue systems occurring in the normal root. The vascular cylinder is surrounded by an abnormally developed parenchymatous tissue which contains the parasite, and in *Alnus serrulata,*

cells containing the fungus are only a little if at all larger than the uninfected ones. No radial elongation of cells could be found as in *Ceanothus*. When mature, the fungus forms a dense cluster in the infected cells, the central portion of which is composed of completely branched threads bearing spherical sporangia at their end. Atkinson considers this fungus as a distinct species from all other forms, and proposes the name *Frankia ceanothi*. A symbiotic relationship between the host and parasite seems probable to Atkinson from the fact that the plant appears to suffer in no respect from the infection.

It was left for Hiltner (16) to prove experimentally that the root tubercles on the alder enable the plant to assimilate the free nitrogen of the air by a process analogous to that which occurs in leguminous plants. Furthermore he showed that alder plants can grow without tubercles provided the solution or soil in which they grow contains nitrogen in some form, and conversely that the growth of the tubercles is inhibited when the plants are grown in a solution or soil where nitrogen is present in abundance. Calcium nitrate entirely stopped the growth of the tubercles.

Subsequent to the above investigation, the attention of Nobbe and Hiltner (28) was called to *Podocarpus,* an oriental conifer, which possesses a large number of root-tubercles caused by an endotrophic mycorrhiza. Guided by their experiment on the alder they carried on investigations with greenhouse plants grown in quartz sand to which only non-nitrogenous culture solutions were added. For five years before their results were published the plants grew luxuriantly and they concluded that they obtained the required nitrogen from the air.

Although Schacht (32), Brunchorst, Möller and a few other earlier investigators had examined the root-tubercle-like structures which are found on the roots of several *Cycas* species grown in green-houses, it was not until 1901 that Life (20) gave a more detailed description of the tubercles, noting that a fungus, a bacterium, and an alga inhabit these structures. In young tubercles and in the tips of older ones, he found only the fungus and bacteria, which, he claims, pre-

pare the way for the blue green alga. Lenticels also occur on the tubercles, which may serve in aerating the root system. Life believed that the tubercles may aid in free nitrogen assimilation for the plant.

Because of the evidence that the fungus living in the tubercles of *Podocarpus, Alnus* and *Elaeagnus,* aids in the fixation of free nitrogen for the use of these plants, Hiltner (17) was led to investigate *Lolium temulentum,* with which, according to Vogel and Nestler, there is associated a fungus that inhabits all the tissues of the plant even including the seed. *Lolium italicum,* on the other hand, contains no fungus. Plants of both species were grown to maturity in nitrogen-containing and nitrogen-free soil with respective controls. Chemical analysis showed that *Lolium temulentum* grown in nitrogen-free sand contained far more nitrogen than was originally present in the seed, and the plants nourished with nitrogen compounds contained nitrogen far in excess of that which was supplied by the solution; hence Hiltner claimed that the free nitrogen of the air is used by this fungus-containing plant. Luxuriant growth of other plants likewise attacked by fungi was considered as evidence in favor of this view, but it has been shown by Brefeld (4) that such is not the case with the Ustilagineae and it remains to be proven for other fungi.

Probably one of the most thorough investigations made on an endotrophic mycorrhiza in relation to its host cell is that of Magnus (22), who sets forth very fully the facts found in the rhizome and root of *Neottia Nidus avis,* where a fungus enters from without and fills a definite concentric layer of cells. All the infected cells do not react alike, for in certain ones the fungus destroys the protoplasm and forms organs for the purpose of maintaining itself during the winter and for the infection of new cells in spring, while in other cells the fungus, after having attained a certain development, is digested for the nourishment of the host, and the undigestable portion remains and collects in a mass in the center or near the cell wall where layers of cellulose are formed around it. The nuclei of host and digestive cells furnish various phenomena which are elaborately described.

Shibata (38) made a cytological study of the endotrophic mycorrhiza found in the tubercles of *Podocarpus chinensis* and *Alnus incana* and in the rhizome of *Psilotum triquetrum*. In the *Podocarpus* tubercles he found a large hyphomycete, which, by branching, filled the entire cell. The host nucleus increases in volume and assumes an amoeboid form, whereupon it divides mitotically, frequently forming as many as eight smaller nuclei which become distributed in the cell and again become amoeboid in form. When the fungus has attained its full growth it is digested by the host cell and the nuclei may resume normal conditions and divide mitotically. No cell walls are formed subsequent to nuclear division, the cells ultimately degenerating with the disintegrating tubercle. Regarding the symbiotic relationship, Shibata corroborates the views held by Magnus (22) and Frank (10), who have shown that the fungus is subservient to the host cell. Shibata showed further by experiment that a proteolytic enzyme capable of digesting fibrin is present in the tubercle.

In the rhizome of *Psilotum* he found the conditions quite similar to those in *Podocarpus*, except that the fungus mycelium confines itself to the periphery of the cell, and the host nucleus undergoes no division. The undigested portions of the fungus form a dense aggregation in the center of the cell where it is cemented together by an amyloid-like substance and finally surrounded by a membrane. This is quite similar to the conditions in *Neottia Nidus avis*. By means of descriptions and figures, Shibata presents the true conditions as they are found in the tubercle of the alder. Here in addition to what earlier investigators had found, he mentions a dense clump of cytoplasm which remains after the absorption of the fungus in which small spherical and heavy stained bodies appear to which he applies the term "Sekretkörperchen," similar to those bodies which Heidenhain found in the gland cells of several animals. These he claims are instrumental in the production of an enzyme in the cell which dissolves the fungus.

Björkenheim (2) in one of the most recent papers on the alder tubercles presents the old facts in a somewhat different

light without, however, contributing anything new. He finds very large hyphae in the young tubercles, some measuring 4–5 μ in width, which become finer as the tubercles grow older. His other facts regarding the fungus and the host cell are similar to those described by earlier investigators.

Wolpert (54) made a study of the tubercles on *Alnus alnobetulina* and corroborates the results of Brunchorst and Shibata, in addition showing intercalary and apical swelling of the septate hyphae, conditions which are similar to the enlarged hyphae found in *Psilotum*. A view not earlier advanced is that the angular structures in the sporanges germinate and form new hyphae, but from the text figures given it would appear to be an artifact rather than an actual germination. The so-called "Secretkörperchen" described by Shibata were not found in those cells where the fungus is digested.

THE TUBERCLES OF CEANOTHUS.

For cytological study the root-tubercles of *Ceanothus americanus* were gathered from plants growing in the open woods surrounding Madison, Wisconsin, the material for subsequent investigations being collected from plants growing in Forest Park, and in other open woods on the outskirts of St. Louis, Missouri. Because the tubercles dry very easily on exposure, fixations were made in the field in order to obtain as nearly perfect material as possible. A few plants, however, were removed from the soil and transplanted into flower pots which were kept in the greenhouses, where the roots were kept in good condition for a reasonable time, packed in a large amount of moist sphagnum.

Judging from the material gathered at various sections of a state as well as from different states the indications are that the species of *Ceanothus* are everywhere affected with tubercle growths. *Ceanothus americanus* and *ovatus,* common in Wisconsin, as well as *azureus, Delilianus, Fendleri* and *microphyllus,* species native to the southwestern United States, which are now growing in the Missouri Botanical Garden, were all examined and found to possess tubercles.

Thus the formation of these tubercles is not confined to any definite locality nor to any special species of the genus.

Of the various fixing solutions used, Kaiser's sublimate-acetic and iridium chloride gave the best results. After fixation the material was hardened and imbedded in the usual manner and transverse and longitudinal sections 5–8 in thickness were made. For staining, Fleming's triple and Heidenhain's iron haematoxylin stains were used, the triple stain proving the better for differentiating the various host tissues, the parts of the cell and the mycelium of the fungus. The nuclear structure of the fungus could best be determined with the iron haematoxylin stain, although the grosser structure, such as the cell wall of the mycelium, is not as distinct. Serial sections prepared with these stains afforded the best means for the study of the minute structure of the fungus and the host, although examinations of fresh material were made in an attempt to obtain pure cultures, and the testing of the enzymes present in the tubercles of course was done with living material. The result of this work is deferred to a subsequent paragraph.

External Characters.

All the plants of *Ceanothus americanus* examined possessed tubercles on their roots; the youngest plants have only a few, often not more than two or three, whereas older plants have a large number; one nine years old possessing 1,830 tubercles on the roots. The number thus increases with the age of the plant and with the root mass, a fact contrary to conditions found in the alder, where very old trees do not seem to have as many as the younger and more rapid growing ones. The tubercles are also found at a greater depth than with the alder, the greatest number and the largest coral-like clusters being usually found 4-10 inches below the surface of the soil. When there are but a few lateral roots the tubercles are frequently found arranged along the tap root, often to a depth of 1.5 to 2 feet, penetrating the hard subsoil, but only individual tubercles or small clusters are found at such depths; the larger masses are found nearer the surface in the looser soil.

It may be noted that the greatest number of tubercles either branched or unbranched are attached by a small root to a larger one, it seldom happening that the tubercles or clusters are attached close to a large root. Often a cluster is formed by a number of rootlets, which are closely packed together, each of which may have several tubercles, so that the irregular masses composed of unbranched individual tubercles are probably caused by the infection of many adjacent rootlets.

The youngest tubercles all originate from the sides of fine thread-like roots (pl. 6), where their beginning may be noted as small protuberances on the sides, infection having probably taken place through an epidermal cell or a root hair. Instances are very rare where a tubercle, or cluster of them, terminates a root so as to result in such massive structures as on the alder. At first the young tubercle is merely a bulging of the epidermal tissue, subsequently the vascular cylinder sends off a branch toward the infected region and the young tubercle becomes spherical, then ovoid and finally elongates into a cylinder as the vascular tissue increases in growth. The average diameter of an ordinary tubercle is from 1 to 1.5 mm. and in one year's growth it may attain from 3–6 mm. in length. The growth in length continues from year to year so that some may become 11–14 mm. long, but they still retain their original diameter. Through di- and tri-chotomous branching the loose cluster is formed on a single rootlet, and one of these may ultimately result in a mass 4–6 cm. in diameter, all of which originated from a single tubercle (pl. 6). There is some variation in the color of the tubercle, due to the nature of the soil in which these plants grow. As a rule the youngest tubercles are light gray, some are almost perfectly white, becoming pinkish as they grow older, while the older ones are flesh colored, becoming darker with age. Atkinson (1) describes the very youngest as having a flesh color, probably due to his not having found the very earliest stages of the tubercles. There is no pigment in the outer layer of cells, such as is abundantly present in the alder tubercles.

Internal Structure.

A study of the internal structure of the tubercle was made from both longitudinal and transverse sections (f. 1), the smallest tubercle being found to possess the various tissues in common with older ones, only in a more rudimentary form. The tubercle consists of the outer corky layer, the outer and inner cortex and the vascular cylinder. Of these tissues the cortical parenchyma makes up the greater bulk of both small and large tubercles, while the vascular cylinder is quite narrow, and does not extend far into the young tubercle (f. 1). The corky layer is made up of several thick walled cells the outer of which are usually broken. Beneath this is a layer of tissue made up of from four to five layers of oblong thick-walled cells, between which and the vascular cylinder is the parenchyma, composed, under normal conditions, of thin walled rounded cells. The vascular cylinder, bounded by an endodermis, consists of a few xylem strands, a large number of phloem cells and some supporting tissue which surrounds both (f. 1). Many resemblances may be noted to the structures of alder, *Elaeagnus* and *Myrica*.

The fungus confines itself to a zone of tissue two or three layers from the cylinder and from eight to ten layers from the outside of the tubercle, an arrangement strictly adhered to in these tubercles, so that no such irregular structure is produced as in the alder. Thus the fungus makes its home in a cylindrical zone extending from the growing point to the base of the tubercle; in older tubercles the infected belt widens considerably, often containing ten to twelve layers of cells in a transverse section, while the vascular cylinder also grows longer and wider and sends a branch into each division of the tubercle.

The developmental stages of the fungus and the effect on the host cell are best studied from a longitudinal section where the various stages, from the apex to the base, are easily recognized, and inasmuch as general cell infection occurs at the apex of the tubercle, the most abundant youngest stages are found in this region. Although internal infection is most prominent in this region it is also found to occur

in other portions of the tubercle as well. From the infected cells containing a mass of mycelial threads a stout hypha passes out, dissolves its way through the cell wall, and passes into the adjacent cell, directing itself toward the cell nucleus, often pushing it aside from its normal position or else twining about it (f. 2). The infecting hypha is usually quite stout, more so than those of the much branched masses (f. 3, 4), a provision evidently necessary because a delicate hypha would not be able to make its way through the wall readily, and even if it did, there would probably not be enough protoplasm present to initiate a good growth in the new host cell.

At this early stage the mycelium is densely filled with protoplasm in which are imbedded many small nuclei showing very prominently with the haematoxylin stain although the walls of the hyphae cannot be seen (f. 5, 6). After having established itself, short branches originate from the first hypha which in turn branch very abundantly and fill the greater part of the host cell (f. 4).

When the fungus enters the cell there is a stimulation to growth of the cell wall and the protoplast; adjacent uninfected cells are comparatively small (f. 8). The cytoplasm increases and becomes densely aggregated about the nucleus or about the fungal material, staining a very light orange, producing a contrast between the cytoplasm of infected and uninfected cells. All traces of starch grains which are abundant in uninfected cells, have entirely disappeared and even normal adjacent cells suffer the loss of their starch and cytoplasmic content from the infection of their neighbors (f. 2). The cell walls of the host are dissolved at quite an early stage and often four or more cells break down, thus increasing the space which the fungus ultimately fills. The original content of such cells is absorbed very quickly. No multinucleated cells are formed in this manner, but the nucleus of the cell in which infection originated grows very rapidly with the fungus and remains the prominent part of the host cell. The nuclei of the adjoining cells, which are thus brought in contact with the infected cell, disintegrate very readily, probably being used as food by the fungus.

Shibata has found multinucleated cells in the tubercles of *Podocarpus* produced by the original nucleus dividing amitotically when thus stimulated by an infecting fungus, but no such condition is apparent in *Ceanothus*. It should also be noted that some cells not directly connected with infected ones have but little protoplasmic content. Either the fungus robs the adjacent cells in some unknown way, or else the infected cells obtain the lion's share of food supplied by the plant and finally starve the other and smaller cells.

As the host cell increases in size the nucleocytoplasmic relationship is maintained and therefore the nucleus increases with the cell, becoming enormously large compared with the nucleus of an uninfected cell. The average diameter of a normal nucleus is about 5.6μ, whereas the hypertrophied nuclei are usually oblong, measuring $14 \times 21\mu$. Some peculiar and amoeba-like nuclei are shown in f. 8, many of which are quite similar to those in *Podocarpus* which Shibata (37) found dividing directly. Even the nucleolus increases its size in relation to the nucleus and in some instances seems even to exceed its natural proportion. The amount of chromatin also increases and it stains a deep color with gentian violet. Passing farther toward the base of the tubercle, the cell and its contents become hypertrophied to the highest degree. In cross section the large infected cells are arranged radially (f. 1), showing in a longitudinal view a more isodiametric form. Those of the transverse section measure $60 \times 113\mu$, whereas those of a longitudinal tangential section are about $50–60\mu$ in diameter. Concurrently with the growth of the cell the mycelium becomes more and more entwined, branching and frequently broken in many places. The mycelial threads now become much finer and the small nuclei are distributed throughout the length (f. 4, 5, 6). This mass of mycelium, however, never becomes so large that it fills the entire cell since a provision must be made for the next following stage in which the pear-shaped vesicles are formed on the ends of the hyphal branches (f. 7). This is confined to a definite region, best seen in the longitudinal section where it stains deeply with gentian violet and very dark with haematoxylin, a fact indicating that there is pres-

ent an abundance of chromatin material in the fungus at this stage. Generally this zone appears in the middle of the young tubercle. The pear-shaped or spherical vesicles, packed closely together around the periphery of the cell, are the swollen ends of hyphae densely filled with protoplasm. In preparing the sections they are often torn loose from the large mass, though still retaining a portion of the hypha (f. 10, 11). They are quite similar both in shape and content to the vesicles found in the tubercle of the alder, except that in the *Ceanothus* form there is no double wall such as is pointed out in the alder by several investigators. At first the vesicles are filled with a dense protoplasm containing one nucleus-like body (f. 10), which at a later stage increase in size. Subsequently the material in the vesicle divides into two parts with a very faintly stained substance between them. Apparently this is the mature condition of the fungus and its ultimate products are contained in the sporange-like structure. Although not surrounded by a distinct and perceptible wall these parts are quite analogous to spores but the final fate of these structures and their relation to the infection of the plant could not be determined.

At this stage every trace of starch and cytoplasm in the host cell has disappeared, its nucleus becoming very irregular and shriveled, staining a light orange and usually lying near the periphery of the fungal mass (f. 7, 9). However, the nucleole is still quite large, its appearance indicating that it retains its vitality. Thus all the protoplasm of the cell is used by the fungus to build up its structure, and not until there is no more protoplasm in the cell does the degeneration of the fungus begin. This is shown by the gradual contracting of the entire fungal mass and the collapse of the walls of the mycelium and vesicles, although occasionally one or two vesicles may be found which are unaffected (f. 13). All signs of nuclei in the fungus disappear and the only thing remaining is the rigid cell wall. At a certain stage in this struggle between the fungus and its host, one would infer that the fungus is the victor, and it may be temporarily, yet its life, after having attained this stage of development, is relatively short and its death is brought about by starvation.

This is quite different from the conditions in the alder (38) and *Neottia Nidus avis* (22), where the fungus is finally absorbed by the so-called digestive cell and the nucleus resumes its natural processes. However, it seems that the normal adjoining cells may utilize, in some manner, the remaining protoplasmic material of the fungus. The turgidity of the host cell becomes less and less and the surrounding cells crowd it into a smaller space, so that none of the earlier structures of the fungus or of the host can be recognized. No nucleus ever reappears, and thus the history of host cell and fungus ends in the death of both. Though Shibata has described, in *Alnus,* certain protoplasmic structures, called "Secretkörperchen," which in some way dissolve the fungus so that it may be utilized as food by the host cell, and Zach (58) has noted similar conditions in *Elaeagnus* as well as in the alder tubercles, nothing of such a nature occurs in *Ceanothus.*

THE ROOT TUBERCLES OF ELAEAGNUS.

Warming (52) was the first to give a full account of the tubercles occurring on the various genera of the *Elaeagnus* family, although he credits Jörgensen with being the first to note the tubercles on the roots of *Elaeagnus, Hippophäe* and *Shepherdia.* Accompanied with a text figure he presents a morphological description of the tubercles. Their cause, he attributes to a parasitic myxomycete resembling *Plasmodiophora brassicae.* The numerous spherical bodies are mentioned and he claims that they resemble spores or are identical with them.

Later Brunchorst (6) made an intensive study of the tubercles of *Elaeagnus angustifolia* and *Hippophäe rhamnoides* in connection with those of *Alnus glutinosa.* He considers the fungus to be the same in both plants and that whatever facts he presents regarding the alder are also true of *Elaeagnus.*

More recently Zach (58) has also made a comparative study of the tubercles on *Elaeagnus angustifolia* and *Alnus glutinosa* in which he points out that the hyphomycete in both plants belongs to the same genus and species: viz: *Frankia*

subtilis Brunchorst. He makes no distinction between the two host cells as they are affected by the fungus, but states that the broken threads, "Stäbchen," so-called by Shibata (38), are the concentrated cell content of the hyphae, the cell wall being unstained. Zach finds spore-like knots and bacteria-like threads which are degenerate forms of hyphae. These he claims absorb a great deal of water and thus fill the entire cell lumen. The terminal swellings of the hyphae are also degenerate stages of the fungus which are ultimately digested by the host cell, during which process the fungal masses pass through various degenerative stages. Spherical, oval and other shaped bodies, of an oily consistency, appear during the digestive process and to these he applies the name "Exkretkörper," though no such bodies as described by Shibata were found.

Zach (60) finds similar conditions in the host cell of the tubercles of *Cycas revoluta,* in which he recognizes a phenomenon comparable to phagocytosis amongst animal cells.

Because the root-tubercles which occur on *Elaeagnus* have been reported by several investigators as resembling in many respects those of the alder, the present investigation was undertaken with *Elaeagnus argentea,* and comparisons were made with the more recent research carried on with alder tubercles as well as those of *Ceanothus* and *Myrica.* The tubercles were gathered in the autumn of 1909 from plants growing in the Missouri Botanical Garden. It was found that they do not occur as abundantly as on the alder and *Ceanothus* and, furthermore, are located on roots much deeper below the surface than in the alder or *Ceanothus.* This, however, may be due to the fact that the *Elaeagnus* has its roots set much deeper in the soil. All plants of this species are more or less infected, yet some have far more than others. *Hippophäe rhamnoides* and *Shepherdia canadensis,* two other representatives of this family, were examined and both found to possess tubercles quite similar to those of *Elaeagnus argentea.* Thus all the genera of this family grown in the garden have tubercles on their roots.

No such large masses or clusters of tubercles as occur on the alder are found, although they vary in size from a few in-

dividual tubercles to much branched structures, the largest of which was 3–5 cm. in diameter. The larger clusters are very compact and usually attached very close to a large root (pl. 7). An unbranched individual tubercle may be described as a cylindrical structure, 8 to 1 mm. in diameter and about 5 to 6 mm. in length, terminating in a smooth rounded tip having no indication of a root cap. Very loose clusters, however, may have tubercles which are much longer. After a tubercle has attained a certain length, branching takes place in the tip which may continue until ultimately 24 to 30 branches are formed, the origin of which may be traced back to a single individual.

The color of the youngest tubercles is a dark gray, becoming much darker near the base. Old tubercles are dark brown or almost black, with a grayish colored tip. This dark color is due, to some extent, to the decaying cortical layer which peels off, thus giving the rough appearance. Individual tubercles of all sizes were fixed in Kaiser's sublimate acetic and Fleming's weaker and medium solutions. Specimens fixed in corrosive sublimate solutions gave the best results; the osmic acid solution so hardened the material that sectioning is difficult. From the imbedded material longitudinal and transverse sections, 5–10μ in thickness, were made, which were stained with Fleming's triple and Heidenhain's iron haematoxylin, but the triple stain set forth clearly all cytoplasmic and nuclear structures of both the host and fungal cells.

The morphological structure of the tubercle is best studied from a median longitudinal section in which the various tissues are shown as well as the different stages of development which the fungus passes through in its limited life cycle. All the tubercles retain to some extent the typical tissue systems of a normal root, the cortical parenchyma being greatly enlarged from the hypertrophy which is primarily brought about by the fungus which lives in the cells. The chief tissues of the tubercle are the inner and outer cortex and the vascular cylinder, which is bounded by a thick walled endodermis (f. 14). The outer cortex consists of oblong cells, the walls of which stain a deeper color with gentian violet, while the

cells of the inner cortex have quite thin walls and are more isodiametric excepting the infected ones which are usually elongated radially. The infected region, as shown in f. 14, is located in the middle portion of the cortex, and the distribution is more uniform than in the alder, being quite similar to conditions in *Ceanothus*. Usually four to five layers of cells outside of the central cylinder remain uninfected, although there is some tendency for the fungus to penetrate inwardly. Many cells are found which are just being infected quite near to the vascular cylinder, but in a young tubercle a comparatively thick layer of uninfected tissue remains outside of the vascular cylinder. In a large mature tubercle the vascular cylinder composes about one-fifth of the diameter and extends quite far into the tip. It is quite uniform in width, except that it tapers somewhat toward the end. Beside the endodermis which surrounds it, the cylinder consists of a few strands of xylem, still somewhat radially arranged, and with phloem cells between them. The bundles are bounded by parenchymatous cells, a number of which fill up the central region of the cylinder.

A study of the developmental stages of the fungus may best be made from a longitudinal section in which the various stages can be determined. Beginning with the apex and continuing toward the base of the tubercle, serial stages may be selected which represent the entire history of the fungus in the various cells, the youngest stages being found in the growing region, whereas the oldest may be noted in cells at the base of the tubercle.

An uninfected host cell is usually isodiametric and contains many large starch grains imbedded in a thin granular cytoplasm. Occasionally a heavy staining substance is met with which, however, has more of an intercellular than intracellular appearance, and is much like the tannin met with in the alder tubercles. The cell nucleus is relatively large and filled with chromatin in a fine reticulated stage (f. 16, 17, 19).

Infection takes place acropetally; the fungus passes from cell to cell by forcing its way through the cell wall (f. 16) and frequently several hyphae may be noted, placed along the inside of the wall for a considerable distance before one

or all pass out of the cell (f. 18). Apparently the fungus secretes some enzyme which dissolves the cellulose wall and thus prepares the way for the infecting hyphae. Upon entering, the fungus directs its hyphae toward the nucleus, then it builds up a dense tangled mass on one side, frequently crowding the nucleus out of its original position. In many of the infected cells the nucleus lies close to the cell wall and the fungal mycelium nearly fills the remaining space. Figure 19 shows where such a condition is just beginning. Again, other stages are found where the nucleus has retained the central position in the cell and the fungus builds its structure entirely about it (f. 16, 19).

When the fungus enters a cell the first noticeable feature is the increase in size of the nucleus, then follow the growth of the cell wall, the disappearance of the grains of starch and the formation of dense cytoplasmic masses in various parts of the cell. The latter may be comparable to that substance in the host cell which Zach (58) calls the "Exkretkörperchen." No indications are found in any of the *Elaeagnus* material examined that large portions of intercellular walls are dissolved in order that a larger space may be provided for the fungus; a phenomenon invariably found in the tubercles of *Ceanothus*. In *Elaeagnus* the cell walls may be more resistant, or when the fungus is once inside the cell it seems as if there are no more secretions which will break down the walls of the host cell. The normal cells adjacent to the infected ones show no signs of being affected in any way by their neighbors at least as far as cell content is concerned.

With the increase in size of the cell and its nucleus, the nucleo-cytoplasmic relationship is constantly maintained. Exact measurements could not be made because of the irregular form of nucleus and the cell; yet measurements as nearly accurate as possible indicate that a definite ratio exists between the size of the nucleus and that of the cell. All nuclei of the infected cells, which at first are spherical often containing several nuclei, are in the so-called resting stage, the chromatin being distributed in very fine masses; frequently this is very dense around the periphery of the nuclear membrane. The

size of the hypertrophied cells varies from 56 to 91×70 to 112μ and the nuclei found in them measure 15 to 20×27μ, while some of the larger spherical ones are only 16 to 20μ, in diameter, though with a nucleolus measuring 5 to 9μ.

At this stage the fungus has attained its greatest vegetative growth and almost fills the entire host cell; a condition which may be comparable to that found by Magnus (22) in *Neottia*. It is likewise at this stage that the host nucleus acquires its greatest volume; the cytoplasm filling every possible space in the cell which is not occupied by the fungus (f. 19). The symbiotic relationship is now very evident, both the host and its guest seeming to prosper for a definite period.

Following the mycelial stage, the ends of the fungal branches begin to swell, forming spherical or pear-shaped bodies which much resemble the fruiting sporanges of a higher fungus. These "vesicles," as they have been called by some investigators, are relatively small, the largest being 2.8 to 3μ wide, whereas the mycelium on which they are borne is only .2 to .3μ in width, and in fresh material the mycelial threads are so delicate that the sporange-like structures break off very readily (f. 19, 20, 21). The young vesicles are filled with granular cytoplasm in which irregular and dark staining bodies may be found (f. 20, 21), and later the content segments into halves, quarters and even smaller portions, in many of which a small nucleus may be determined. This process is quite analogous to spore formation among higher types of fungi, yet no stage can be found where there is a definite rounding up of the spore-like mass into structures with definite walls. The formation of walls may be inhibited because of the parasitic nature of the fungus, or the fungus may be of that primitive type where no thick wall is formed around the spore.

It is at this stage that the host nucleus assumes an amoeboid shape, frequently becoming very irregular, and portions project quite a distance into the fungal mass (f. 19, 25), a condition probably due to lack of space in which to round out more uniformly. This condition gives some indication of the host cell assuming a digestive function although not

so effective as the digestive cells of *Alnus,* orchids, and *Podocarpus.*

After the development of the sporanges the fungus, as an entire mass, begins to collapse, the sporanges break open and their walls, which stain dark with the triple and haematoxylin, appear as mere shell-like coverings. All of the indications are in favor of the view that the content of these sporanges has escaped but whether in the form of a swarm spore or otherwise could not be determined. When the fungus is in this stage the host cells contain very irregular nuclei (f. 26), and serial stages may be noted where the nuclear content gradually dwindles away until finally nothing but the nucleolus and the nuclear membrane remain; and these too ultimately disappear. Thus there is a long continued struggle between the host and the fungus resulting finally in the destruction of the cell, the nucleus being its most resistant part. The question may arise whether the cell might not still be living after the nucleus is destroyed; all indications are negative, however, being quite similar to those which Gerassimoff (11) obtained with enucleated *Spirogyra* cells which had but a short life even though the cytoplasm was left in almost a normal condition. Just how far the fungus and the host have been mutually injurious, and which one outlives the other, is a difficult question to answer. If the destruction of the nucleus signifies the ceasing of cell activity, then the host cell is destroyed by the fungus which in turn dies from starvation. According to Zach (60) the host cell destroys or digests the fungus, leaving but a portion of undigestible material to which he applies the term "Exkretkörper," but no statement is made as to what the cell does after it has gone through this digestive process. Similar conditions are found in the tubercles of *Cycas,* but here Zach points out that both host cell and fungus may be destroyed. Certainly in *Elaeagnus* the host cell and the fungus both die as a result of their relationship and there is no indication of such a perfect symbiosis as occurs in the alder and *Podocarpus,* although it may be true that the normal cells surrounding the infected ones derive some slight benefit from the fungus. Long after the apparent de-

struction of the host cell the fungus gradually loses its content until finally nothing remains but the walls of the hyphae.

Granting the fact that the host cell is destroyed before the fungus, the gradual disappearance of the latter must be caused by agencies outside of that particular cell, for if it were but a ceasing of living conditions the various stainable parts of the fungus would remain in the cell for a long time. However, numerous examinations of cells of this kind from the oldest portions of the tubercle show only walls closely packed together by the collapsing of the host cells where they undergo no further change (f. 23). Whatever benefit the host plant derives from the fungus must be obtained through the host cell while it is yet in a living condition, or by the other living cells which adjoin the infected ones. Possibly the plant can acquire greater gain by suffering the loss of a few cells for the good of many.

THE TUBERCLES OF MYRICA.

The amount of research that has been done on the tubercles of *Myrica* is rather limited when it is compared with all that may be found on the alder; due probably to the fact that they were considered, for some time, to be caused by a similar fungus. Brunchorst (6) was the first to mention the tubercles on *Myrica Gale* and considered the fungus producing them so much like that in the alder that he called it *Frankia subtilis.* Later, Möller (26) found that it differed considerably and made it a new species which he called *Frankia Brunchorstii.*

Shibata (38) has made the most thorough investigation on the root-tubercles of *Myrica rubra,* his observations being on fresh material and on some prepared according to modern cytological technique. He describes the external and internal morphology of the tubercle, noting that the fungus confines itself to a definite region, thus differing from the condition found in the alder and other forms. The differentiation of the tissues begins in the meristematic region, where internal infection of the young cells takes place. At first the fungus consists of a few mycelial threads which give rise to

branches arranged in an aster-like form and ultimately filling the entire cell. Shibata places this fungus with the genus *Actinomyces,* thus differing from that found in the *Podocarpus, Psilotum, Alnus* and *Elaeagnus* tubercles.

Harshberger (15) was the first to report the tuberculous outgrowths on adventitious roots of *Myrica cerifera,* although Brunchorst and Shibata had already made some investigations on *Myrica Gale* and *M. rubra.* He calls them mycodomatia, a term used by Tubeuf (52) for these structures. The tubercles are found on these roots when the stems are surrounded by shifting sand. At first the masses are relatively simple, but by continual branching and growth, aggregations attaining the size of a walnut are produced. The structure of the tubercle was studied from dry material which had been boiled with water and then treated with alcohol. From sections obtained from these he describes the various parts of the tubercle, finding a unicellular hyphomycete, which confines itself to a definite region, infecting cells anew by passing through their walls and forming a dense mass of hyphae within. However, the microphotographs obtained from these sections do not show the true nature of the fungus and Harshberger, rather unwarrantedly, claims for the fungus a position closely related to the *Oomycetes.*

The root-tubercle-like structures on *Myrica cerifera* were gathered in November and December on plants growing in the Missouri Botanical Garden. *Myrica Gale* and *M. asplenifolia* were also examined, but these did not afford as large or as abundant material as the above named species. These structures occur on adventitious roots which grow out from the lower part of the stem, or from branches or stems which have been covered over with leaf mould or soil for several years. This fact agrees with the observations of Harshberger (14), who found these growths on stems which were surrounded by sand in localities where sand dunes are formed. Other roots have a few tubercles which, however, do not attain so large size as the ones that occur on the adventitious roots, nor are they as abundant as the tubercles found on *Alnus, Ceanothus* and *Elaeagnus.*

The tubercles are usually found in masses varying in size

from a pea to that of a walnut (pl. 8). Specimens of the large size were always associated with large stems. Those found on stems three years old had attained a mass 1 to 1.5 cm. in diameter. The cluster is usually very compact and resembles somewhat that of the alder and *Elaeagnus,* although differing in this respect from *Ceanothus.* Harshberger claims that the tubercles are of very slow growth and tries to determine the age of a cluster by comparing it with the age of the plant on which it is found, but his estimate can be only relative. The masses shown in the photograph (pl. 8), growing on plants three years old, have attained 2 cm. in diameter. According to his statement some plants were twenty years old. Granting that growth be uniform, and if three years' growth will produce a mass 2 cm. in diameter, on a plant twenty years old, one would expect a cluster a half a foot in diameter, a size not attained on any plant observed by either Harshberger or myself.

The individual tubercle may be described as a short thickened rootlike structure which branches di- or trichotomously after having attained a certain length. The longest individual branches found were 2 mm. in length. Their thickness varies from .5 to 1 mm., the older portions, however, being the thickest. One peculiarity of these tubercles is that, after having attained a certain length, their tips grow out into a narrow thread-like structure, often attaining 1.5 to 3 cm. in length (f. 28). This again sends out lateral branches which may be found entwined amongst the roots and grass blades. In vigorous growing material the outgrowths of this kind appear quite like ordinary roots of the plant except that their shape is more tapering toward the tip. The structure of the tubercle will be described in detail in a subsequent paragraph.

The color of the youngest tubercles is a light gray to pink changing to a flesh color with age. The long slender tips, nearly colorless when young, become brown as they dry, through exposure to the air and soil. The very old and dry tubercles are dark brown or even black, a color which was attributed to them by Harshberger, who made his investigation with dried material only.

The tubercles originate from the small adventitious roots which do not attain more than several centimeters in length when infection takes place. Many roothairs occur on these roots, and it is quite probable that the fungus, in some form, makes its inroad through them or by some epidermal cell of the growing root. The formation of a mass of tubercles ends the growth of that particular root, where probably the food material all passes into the tubercles.

The material for investigation was taken from living plants, and after a thorough washing was fixed in various solutions, of which Kaiser's sublimate-acetic and Fleming's weaker fluid proved to be the better fixative for these structures. After fixation, the ordinary procedure of dehydrating, hardening and imbedding was followed, though a much longer time was given for infiltration than is usually given for tissues of a softer texture. Tangential, median and transverse sections 5 to 6μ in thickness were made and stained with Fleming's triple and Heidenhain's iron haematoxylin. The haematoxylin stains the nuclear structures of the fungus, but the mycelial wall and different parts of the host cell can be made out with difficulty. Most of the drawings were made from sections prepared only with the triple stain.

The various tissues which compose the tubercle may best be studied from a transverse section. The tubercle is covered on the outside by an epidermis which is usually broken and very irregular except in very young forms. Underneath lies a thick layer of cortex, made up of rather thin walled parenchymatous cells. The outer portion of this contains narrow oblong cells which make up a layer from 4 to 5 cells in thickness. Inside of this there is a layer of cells almost isodiametric, with very thin walls. Farther toward the interior, the cells become larger and radially elongated, some measuring 20 to 25 $\times$ 28 to 45μ. These cells contain the parasitic fungus, forming a definite region two or three cell layers in thickness. Adjoining it on the inside is a region of smaller cells mostly isodiametric with a few oblong ones scattered irregularly amongst them.

The vascular cylinder is bounded by an endodermis composed of small oval thick-walled cells which stain quite

deeply with the gentian violet. The structure of the vascular cylinder does not differ materially from that of the normal root. In the young tubercles the xylem and phloem are arranged alternately and radially, with a few pith cells in the center. The phloem, however, makes up the greater bulk of the tissue.

In the young tubercles the vascular cylinder does not extend far into the apex, whereas in older ones the cylinder with some cortical cells surrounding it, grows out into a slender thread from which lateral branches are again sent off. These, no doubt, have some absorbtive function similar to that of the ordinary root.

The fungus which lives in these tubercles may best be studied, in its various relations, in a median longitudinal section where the youngest stages may be found in or near the meristematic region and the older stages may be traced back toward the base of the tubercle. The infecting region is confined to the apex of the tubercle, indicating that only embryonic or comparatively young cells afford the proper conditions for the fungus. There are numberless cells adjoining, but none of them show indications of being affected. This layer is shown in f. 29. The entire infected region has the shape of a cylinder, ranging from one to two cell layers in thickness, and tapers slightly toward the apex of the tubercle where it always remains open. This region may be recognized from the place where the tubercle begins to taper. Such an arrangement seems to be indicative of the fact that the fungus has certain selective properties in determining where it may best grow in the host tissue. Another strange feature is the fact that it will live in one or two layers of cells, growing neither farther out nor in. Probably the cells have acquired some immunity after having attained a certain age, or the infected region may bear some relation to the central cylinder and also to the air outside of the tubercle and thus the fungus may have selected the most appropriate position.

The young uninfected embryonic cells are usually filled with starch grains imbedded in a spongy cytoplasm in which frequently a large vacuole is present. The starch grains are

large and simple, but occasionally compound grains may be found in the older cells. The cell nucleus is spherical in shape and relatively small compared with the size of the cell (f. 30). The chromatin stains a deep blue and the greatest number of nuclei are found in the so-called resting condition, indicating that the cells are quite active in their metabolic process.

Internal infection is accomplished by the fungus passing from cell to cell, penetrating the cell wall, and as the mycelium is very thick, frequently 1.4 to 2μ at this stage, it must secrete an enzyme which is capable of dissolving the cell walls so as to allow three or more parallel strands of hyphae to pass into a cell (f. 32). The fungus directs itself toward the cell nucleus, in the neighborhood of which it must derive its greatest benefit. Subsequent to this stage, the mycelium grows very rapidly and begins to branch and coil itself on one side of the nucleus, which is frequently crowded to the wall; occasionally, however, instances are found where the nucleus is contained within the fungal mass.

Although this fungus is considered a parasite there are no indications of such relationship as exist between the haustorium of the mildews and the nucleus of their respective host cells where it has been shown that the nucleus becomes very irregular and surrounds closely the haustorium, in which condition both nucleus and cytoplasm are ultimately absorbed by the fungus. Another singular feature is the fact that there is no apparent hypertrophy of the host cell, such as occurs so commonly among cells infected by the rusts. Apparently the host cell of *Myrica* affords less resistance to its fungus than the host cells of various plants offer to their respective mildews and rusts; even the host cells of *Ceanothus* and *Elaeagnus* display a greater resistive power.

The host cell undergoes but few changes after the fungus makes its entrance. First the starch grains, which are used as food, disappear, the cytoplasm of the host cell, which at first increases, gradually becomes less dense and finally vanishes (f. 33, 37). Comparative measurements were made of the nuclei and cells of infected and neighboring uninfected regions, and it was found that there is no increase in

size due to infection. Thus there is no expression of such a symbiotic relationship as is found in so many of the other plants, but the condition is rather one of real parasitism, where the fungus obtains the full benefit from the cells in which it lives. However, the host nucleus offers a great resistance, retaining its natural content and form for a long time. Even in some very old infected cells the nucleus may be found in nearly perfect condition; although ultimately the nuclei begin to collapse and are found as small shriveled bodies with the dead mycelium. The cell wall remains quite firm, being held in position, to some extent, by the adjoining rigid walls of other cells.

Passing farther toward the base of a tubercle, the cells are found filled with the fungus. A minute study reveals that the mycelium is composed of dense granular protoplasm in which are imbedded many nuclei of considerable size (f. 34). No cross walls can be distinguished. After a cell becomes partly filled with the mycelium, branches are sent out radially toward the periphery of the cell, which at first are narrow and oblong, but finally swell at the ends forming club or wedge shaped structures as seen in a median section (f. 35, 36). The largest ones are 3.5 to 4μ across at the widest part and usually 7 to 10μ long. For a considerable distance back from the apex the vesicles and hyphae are filled with a dense granular protoplasm in which may be found a few nuclei, while in older stages the hyphae and vesicles are empty. Thus the fungus dies out gradually, death being due to lack of food material after this has all been absorbed.

The question arises whether the plant, in an indirect way, derives a benefit from having the fungus living in its tissues. From all appearances no specific injury can be noted. It may be that the fungus can use and change the protoplasm of the host cell in such a manner that it can be taken up again from the fungus by the adjoining uninfected cells; or the conditions may be comparable to those which Hiltner (17) finds in *Lolium temulentum,* where the fungus utilizes the free nitrogen of the air which in turn is taken up by the plant. The position which the fungus selects in the

tubercle is indicative of some relationship with the atmosphere, but this fact can only be proved by accurate experiments.

It is somewhat difficult to place this fungus systematically when one can judge its morphological characteristics only from the form found within the host cells. Harshberger (15) favors the view of locating it among the Oomycetes, but judging from the characteristics as noted, it cannot properly be considered to belong to that group. Shibata (38) is probably correct in placing it with *Actinomyces* as defined by Migula in his System of Bacteriology. Pecklo (29), in his pure culture of endotrophic mycorrhiza, claims to have isolated an *Actinomyces*-like fungus from the tubercles of *Myrica Gale,* apparently the only instance of actinomycosis that has ever been reported amongst plants. In the 302 references on *Actinomyces,* given in Kolle and Wassermann's Handbuch der pathogenen Mikro-organismen (19), no mention is made of such organisms infecting plant cells, yet a large number of these pathogenic forms, in the early stage of their life history, live in the intercellular spaces of various plant tissues and several investigators have obtained pure culture of *Actinomyces* from awns of barley and grasses, so that it is not altogether improbable that a similar form may inhabit the root tubercles of *Myrica.*

COMPARISON AND DISCUSSION.

Although the tubercles and fungus on *Ceanothus, Elaeagnus* and *Myrica* have been considered the same or quite similar by several investigators, a comparative study shows that a number of variations may be noted in the fungus as well as the structure which it produces. It may be noted that the tubercles on the above plants have very little in common with those of bacterial origin found on the Leguminosae.

Differences regarding the number and location of the tubercles as found on these plants may be noted as follows: On the roots of *Elaeagnus* and *Myrica* they are not so plentiful as on *Ceanothus.* On *Myrica cerifera* they occur on the short adventitious roots which grow out from the lower part

of the stem or procumbent branches. This appears to be characteristic of this species only, for the other two species possess the tubercles on ordinary roots, like *Ceanothus.* The largest and most compact masses of tubercles occur on *Elaeagnus,* yet many may be found on *Ceanothus* which have attained nearly the same size. The dense collection of tubercles of *Myrica* is due more to the intricate interlacing of the branches than to the close proximity of the individual tubercles. The most striking external difference between the tubercles of *Elaeagnus* and *Ceanothus* is their color. The former are very dark, due to the black cortical layer which peels off and is changed to a darker color, probably by the oxidizing agents of the air and soil. In *Ceanothus* the young growing tubercles are almost colorless, but later they assume a flesh color which turns still darker as they become older. In *Myrica* the color varies from glistening white growths to the brown or almost black mature structures. As to form, those of *Ceanothus* and *Elaeagnus* are quite similar. The former attain by far the greater length, but do not form so thick or irregular a growth as in *Elaeagnus.* However, there seems to be more symmetry in the growth and branching of those of *Ceanothus* than in any other type. A great deviation from those described is found in the *Myrica* forms. Their branching is far more irregular and abundant. It is difficult to compare them as to length and thickness. One peculiar feature is the elongation of the tip which forms a long slender thread in which may be found the central cylinder. No indication of such a growth is found among the other tubercles, however great a length they may attain.

All the foregoing facts may be but minor differences, due, more or less, to environmental conditions in which the plant finds itself. The more important variations may be noted in the fungus of each host with its various tissues. In the tubercles of *Elaeagnus* and *Ceanothus* the fungus shows some similarity in behavior. In both a definite region of the cortex is infected, the mode of infection does not differ very materially. One variation exists in the fact that, in *Elaeagnus,* the fungus is unable to dissolve, either directly or indirectly, portions of cell walls after it has entered and

established itself inside the cell. In *Ceanothus,* however, the growth of the cell ceases at a certain stage, but as the fungal mass enlarges, the cell walls are dissolved and thus more space is provided. Several differences may be noted in the minute structure of the fungus of each of these plants. The mycelium of that in *Ceanothus* measures from 1 to 1.4 μ in width, is septate and has branches arising at irregular intervals. That of *Elaeagnus* is very fine, being but .2 to .5 μ at its widest parts, becoming much branched and entwined. Although this is true, the vesicles produced on both are of the same size, and the breaking up of their content and its subsequent fate does not differ very much.

The nuclei of the fungus in *Elaeagnus* are very much smaller and more numerous than those found in *Ceanothus* and *Myrica,* yet they are quite distinctly differentiated from the cytoplasm with the safranin, a result difficult to obtain with that of *Ceanothus* by using similar methods. Accurate measurements could not be made because none of the nuclei are more than a small fraction of a micron in diameter.

In both forms the content of the sporanges breaks up into several segments so that frequently four or more can be seen in one plane; but in *Ceanothus* the number of segments is smaller. In both, a close resemblance may be noted to conditions which Shibata (38) points out in the fungus of the alder where a large number of segments are produced in one sporange. These facts cannot be found in the fungus of *Myrica* tubercles. No differentiation of the content in the vesicles takes place even after the nuclei have wandered into them. This and other features of this unicellular fungus indicate that it is entirely different from that in *Ceanothus* and *Elaeagnus.*

No such perfect symbiosis between the host and its fungus can be attributed to *Myrica* as has been found in *Podocarpus* and in all the other forms that have been mentioned. If the fungus were more closely associated with the exterior of the tubercle, Stahl's views, that it absorbs mineral salts and changes them into nitrogenous compounds for the use of the plant, might offer some solution of the problem involved. This may be true among the ectotrophic mycorrhiza, but in

the forms under consideration it is rather difficult to determine how closely the fungus is related to the external environment. The host and fungal relationship may be slightly symbiotic from the fact that only a few cells are infected, yet a large number of cells is produced by the fungal stimulation, this excess of growth being easily noted just at the region where internal infection takes place. No hyperchromatic cells, so common in other tubercles, are produced in *Myrica.* As has been previously stated, the host cells of the three forms studied are destroyed as a result of their association with the fungus. In *Myrica* the problem regarding the function of the fungus in the tubercle is still unsolved. In *Ceanothus* and *Elaeagnus,* on the other hand, the evidence that the fungus is digested in part by the host cell indicates that the plant may derive some benefit from the fungus. The statement is frequently met with that the fungal organism in *Elaeagnus* enables the plant to utilize the free nitrogen of the air. This must be based on an analogy with the alder, for I have found no experimental data of any investigator which will substantiate such an assertion.

Zach (55, 57) lays great stress upon the digestive activity of the host cell in the several forms which he has studied, and attributes to it functions similar to those of the phagocytes found in animals. Even though the fungus is destroyed in part by the cytoplasm of the cell, the above analogy does not explain the actual processes which take place when the fungus is destroyed.

Regarding its systematic position, the fungus in the tubercles of *Ceanothus* and *Elaeagnus* must be retained within the genus *Frankia,* respectively as *Frankia subtilis* Brunchorst, and *Frankia ceanothi* Atkinson. The fungus found in *Myrica,* as already pointed out, has but a few minor characteristics in common with the species found in *Ceanothus* and *Elaeagnus,* and should probably be placed in a separate genus. The name *Frankia Brunchorstii* serves the purpose of designating it only by a great extension of the generic characters of *Frankia,* its *Actinomyces* nature making it quite distinct from the other species.

ENZYMES IN ALNUS AND CEANOTHUS TUBERCLES.

The experiment of Shibata (38) demonstrating the presence of proteolytic enzymes in the tubercles of *Podocarpus* and to some extent in the alder, led me to carry on similar experiments with the tubercles of the alder and *Ceanothus*.

Three different kinds of extracts were made from the tubercles: in glycerin, distilled water, and 1% NaCl. solution. The best reactions were obtained with the aqueous and glycerin extracts. The NaCl. solution gave no decisive reactions, probably due to the toxic effect which this salt has upon the enzyme. Several controls were carried on with root extracts, distilled water and glycerin. To every gram of tubercle 30 grams of solution was added. This was mixed and finely crushed in a mortar and filtered after having stood for an hour. The material crushed with glycerin was left standing for ten to twelve days, after which it was filtered through fine fabric and the filtrate was diluted with four parts of distilled water. The extracts were placed in sterilized flasks and the weighed portion of blood fibrin was added after being soaked in a 1% solution of HCl. A small drop of chloroform was added to all the extracts of each flask as an antiseptic against fungi and bacteria.

Experiment I.

To 50 cc. of diluted glycerin extract of alder tubercles .25 gm. of dry fibrin was added, and it was kept at a temperature of 34° C.

1. A normal glycerin solution.
   (a) At end of 24 hrs.—Somewhat digested.
   (b) " " " 48 " Two-thirds of fibrin digested.
   (c) " " " 72 " Nearly all digested.

2. Glycerin extract with 5 cc. of 1% HCl.
   (a) At end of 24 hrs.—Half of the fibrin digested, the liquid turbid with many bubbles of gas.
   (b) At end of 48 hrs.—Only a few small pieces of fibrin left.
   (c) " " " 72 " All the fibrin digested.

The solution gave a good biuret test for proteids.

3. Extract which had been heated for ten minutes at 80° C.

(a) At end of 24 hrs.—No digestion, liquid clear.
(b) " " " 48 " No action, " "
(c) " " " 72 " Fibrin still unaltered.

4. The control, with glycerin and aqueous extracts of root tissue, was placed under the same conditions as the above, but no indication of digestion was shown.

Experiment II.

Distilled water extract was made of alder tubercles. To 50 cc. of extract, .25 gram of fibrin was added. Temperature 34° C.

1. A normal aqueous extract.

(a) At end of 48 hrs.—Half of fibrin digested.
(b) " " " 72 " More digested, liquid turbid.
(c) " " " 96 " All digested.

2. Extract with 5 cc. of 1% HCl.

(a) At end of 48 hrs.—Considerable digested.
(b) " " " 72 " Fibrin nearly digested, liquid turbid.
(c) " " " 96 " Fibrin all digested.

3. Extract with 5 cc. 1% $Na_2 CO_3$.

(a) At end of 48 hrs.—Liquid clear, no digestion.
(b) " " " 72 " Fibrin hardened, no digestion.
(c) " " " 96 " No digestion.

4. Extract heated to 80° C.

(a) At end of 48 hrs.—No reaction.
(b) " " " 72 " No reaction.
(c) " " " 96 " No change in fibrin.

Experiment III.

Glycerin extract of alder root tissue. To 50 cc. of extract .25 gram of fibrin was added. Temperature 34° C.

1. Normal extract.

(a) At end of 24 hrs.—No reaction.
(b) " " " 48 " Fibrin shriveled.
(c) " " " 72 " No digestion.
(d) " " " 96 " No reaction for digestion.

2. Extract with 5 cc. 1%HCl.

No change in fibrin could be noted at end of 96 hours, and the filtered liquid gave only a slight biuret test for proteids.

3. Extract with 5 cc. 1% Na_2CO_3.

No reaction for proteids at the end of 96 hours.

4. Extract heated to 80° C.

No digestion of fibrin could be noted.

The above data indicate that the greatest amount of fibrin is digested in a weak acid solution and at the higher temperature. No loss of weight of the fibrin was obtained with alkaline, heated and root tissue extracts.

Experiment IV

An aqueous extract of alder tubercles was used to determine the amount of fibrin 50 cc. will digest in 24 hours at 20° and 43° C. The fibrin was thoroughly dried before each weighing and then soaked in 1% HCl. before it was used.

Extract.	Original wt.	After 24 hrs.	Loss.
	At 20° C.		
1. Normal.	.25 g.	.249 g.	.001 g.
2. With 5 cc. HCl. (1%).	.25 "	.23 "	.02 "
3. With 5 cc. Na_2CO_3 (1%).	.25 "	.25 "	No loss.
4. Heated to 80° C.	.25 "	.25 "	No loss.
	At 43° C.		
1. Normal.	.25 g.	.217 g.	.033 g.
2. With 5 cc. HCl. (1%).	.25 "	.115 "	.135 "
3. With 5 cc. Na_2CO_3.	.25 "	.249 "	.001 "
4. Heated to 80° C.	.25 "	.25 "	No loss.

Experiment V.

With extract from root tissue.

Extract.	Original wt.	After 24 hrs.	Loss.
	At 20° C.		
1. Normal.	.25 g.	.25 g.	None.
2. With 5 cc. 1% HCl.	.25 "	.25 "	None.
3. With 5 cc. 1% Na_2CO_3.	.25 "	.25 "	None.
4. Heated to 80° C.	.25 "	.25 "	None.

Similar experiments were carried on with root extract at 43° C., but no loss of weight was obtained.

Experiment VI.

With aqueous extract from *Ceanothus* tubercles, .25 gram of fibrin was placed in 50 cc. of extract which was kept at 33° C. A drop of chloroform was added as an antiseptic.

Extract.	At end of 7 hrs.	After 24 hrs.
1. Normal.	Slight action—liquid becoming turbid.	Some digested.
2. With 5 cc. 1% HCl.	Apparent digestive action.	Fibrin becoming filled with bubbles.
3. With 5 cc. Na_2CO_3.	Liquid dark, fibrin contracted.	No action on fibrin.
4. Heated to 70° C.	No action.	A slight precipitate.

The same extract.

	At end of 48 hrs.	After 72 hrs.
1. Normal.	Fibrin digested, liquid turbid.	Half of fibrin digested.
2. With 5 cc. 1% HCl.	One-half of fibrin digested.	Only a few small pieces left.
3. With 5 cc. 1% Na_2CO_3.	No digestion.	No digestion.
4. Heated to 70° C.	No action.	No action.

After 48 hours of digestion some filtered liquid from each flask was tested for proteid. The solutions from 1 and 2 gave good biuret tests, indicating further that normal and acid extract will digest fibrin. No proteid reaction could be obtained from 3 and 4, showing that the enzyme was destroyed under the conditions or else its action was inhibited. After six days all the fibrin in 1 and 2 was digested.

Experiment VII.

To 50 ccc. of dilute glycerin extract of root tissue of *Ceanothus* .25 gram of fibrin was added. A drop of chloroform was added as an antiseptic. Temperature was 23° C.

Extract.	After 7 hrs.	24 hrs.	72 hrs.
1. Normal.	Liquid clear.	No digestion.	No digestion.
2. With 5 cc. 1% HCl.	No digestion.	No digestion.	No digestion.
3. With 5cc. 1% Na_2CO_3.	No digestion.	No digestion.	No digestion.
4. Heated to 80° C.	No digestion.	No digestion.	No digestion.

Judging from these results no enzyme which will digest fibrin is present in the root tissue of *Ceanothus*.

Experiment VIII.

To 50 cc. of dilute glycerin extract of *Ceanothus* tubercles .25 gram of fibrin was added. A drop of chloroform was added to each flask as an antiseptic. The temperature was 22°–23° C.

Extract.	After 14 hrs.	After 24 hrs.
1. Normal.	No action, fibrin very loose.	No digestion.
2. With 5 cc. 1% HCl.	No digestion.	Slight digestion.
3. With 5 cc. 1% Na_2CO_3.	No digestion.	No change of fibrin.
4. Heated to 80° C.	A slight precipitate.	No digestion.

Extract.	After 48 hrs.	After 72 hrs.
1. Normal.	Some digestion.	Considerable digestion.
2. With 5 cc. 1% HCl.	Liquid turbid.	All digested.
3. With 5 cc. 1% Na_2CO_3.	No change of fibrin.	No reaction.
4. Heated to 80° C.	No digestion.	No reaction.

Extract.	After 96 hours.
1. Normal.	Only a few pieces undigested.
2. With 5 cc. 1% HCl.	All digested.
3. With 5 cc. Na_2CO_3.	No reaction.
4. Heated to 80° C.	No reaction.

Two other experiments with a similar extract were carried on at 33° and 43° C. At 33° C. the results were similar to those which were obtained at 23° C., whereas at 43° C. the digestive activity was much slower. Hence the optimum temperature for the enzyme is lower than 43° C. At 23° C. there is no perceptible digestive action during the first thirty-six hours, but following this period, the process goes on very rapidly and in the normal and acid extract the fibrin is readily digested. No fibrin was digested in the alkaline extract or in that which was heated to 80° C. Even 60° will stop the action of the enzyme.

If my interpretation of the preceding data be correct there is present, in the tubercles of the alder and *Ceanothus*, an enzyme capable of digesting fibrin. The enzyme obtained from the *Ceanothus* tubercles is more active at a lower temperature than that from the alder which digests more readily at a higher temperature. The enzyme, however, is found

only in the tubercles, for experiments with root tissues show that it is not present in the normal root.

The question still presents itself whether the enzyme is produced by the host cell or by the fungus. Judging from cytological data, the digesting of the fungus is an indication that the host cell produces an enzyme. The dissolving of the cell walls in the tubercles of *Ceanothus* presents a fact which indicates that the fungus also produces an enzyme. Thus there may be two enzymes present, one produced by the host cell and another by the fungus, for it is hardly probable that the host cell forms an enzyme which dissolves its own walls. Until a sufficient amount of pure culture of the fungus can be grown, it is impossible to decide whether the fungus secretes an enzyme or not, but the conditions are probably quite similar to those which Marshall Ward found in *Botrytis,* which produces cytase capable of dissolving the walls of the host cell.

SUMMARY OF RESULTS.

The Tubercles of *Ceanothus.*

1. Judging from the common occurrence of the tubercles, the infection of this plant by the fungus is quite universal. 2. External infection probably takes place through a root hair or an epidermal cell from which the subsequent tubercle is formed. 3. The tubercle consists of three systems of tissues: the outer or corky layer; the inner, the vascular cylinder; and the middle or cortex, which contains the infected cells. 4. Internal infection occurs in the growing region and takes place by the fungus passing from cell to cell. 5. Three distinct stages of fungal development may be noted: the mycelia stage found in the host cell; the stage with the sporanges, which initiates the conditions for the digestive cell; and the last stage, where all but the walls of the mycelium are absorbed. 6. Because of infection, hypertrophied cells and nuclei are formed. The fungus dissolves the walls of the host cell. 7. The host nucleus increases in volume; with it, there is an increase of the nucleole and in the amount of chromatin. 8. Following the vesicular stage the cytoplasm and nucleus of the host cell are absorbed. Subsequent to this, the cell content of the fungus disappears. 9. Both the host cell and the fungus finally die and undissolved portions of the fungus remain in the cell. 10. Symbiosis exists, which is quite apparent in the early stage.

Elaeagnus.

11. The tubercles are not found as abundantly as on *Ceanothus*. Regarding the form and structure, several resemblances can be noted. 12. External and internal infection takes place as in *Ceanothus*. 13. The fungal mycelium differs from that of *Ceanothus* in being very narrow. It branches profusely, forms the vesicles, the content of which breaks up into several segments. The infected cell passes through various stages. The fungus is not entirely absorbed by the digestive cell. 14. The walls of the host cell are not broken down as a result of the fungal infection. 15. Hypertrophied cells and nuclei are formed, but the nucleo-cytoplasmic relationship is maintained in the infected cells. 16. No "Exkretkörperchen," such as Zach reports, can be found in the digested cells. 17. Both the host cells and the fungus die as a result of their previous relationship.

Myrica.

18. The tubercles and fungus of *Myrica* differ in many respects from those of *Ceanothus* and *Elaeagnus*. All species of *Myrica* possess tubercles. 19. The fungus confines itself to one or two layers of cells and internal infection takes place acropetally. No hypertrophy or symbiotic relationship exists. The fungus is best regarded as a parasite. 20. The unicellular hyphae of the fungus form branches which change to club-shaped structures in which no further differentiation takes place. 21. The fate of the host cell and fungus is similar to that in *Ceanothus*. 22. The form, structure and behavior of the fungus indicate that it belongs to the genus *Actinomyces*.

Enzymes.

23. In the tubercles of the alder and *Ceanothus* enzymes are present capable of digesting fibrin. Whether two enzymes are present, one produced by the host and another by the fungus, could not be determined without a pure culture of the fungus.

INDEX OF LITERATURE.

1. Atkinson, G. F. '92. The genus *Frankia* in the United States, (Bull. Torrey Bot. Club. **19**: 171–177).
2. Björenheim, C. G. '04. Beiträge zur Kenntnis des Pilzes in den Wurzelanschwellungen von *Alnus incana*. (Zeitschrift für Pflanzenkrankheiten. **14**: 128–134).
3. Bourquelot, M. E. et H. Hérissey. '95. Les ferments solubles de *Polyporus sulphureus*. (Bull. Soc. Myc. de France. **11**: 235–240).
4. Brefeld, C. '02. Versuche über die Stickstoffaufnahme bei den Pflanzen. (Centrb. für Bakt. u. Parasitenkunde. **28**: 24–25).

5. Brown, Escombe. '98. On the depletion of the endosperm of *Hordeum vulgare* during germination. (Proc. Roy. Soc. Lond. **63**: 3).

6. Brunchorst, J. '86. Über einige Wurzelanchwellungen, besonders diejenigen von *Alnus* and Elaeagnaceen. (Unters. bot. Inst. Tübingen. **2**: 151–177).

7. Burgeff, H. '09. Die Wurzelpilze der Orchideen und ihr Leben in der Pflanze. Jena.

8. Döbner, E. P. '53. Lehrbuch der Botanik für Forstmänner. 3 ed. 71.

9. Frank, B. '80. Die Krankheiten der Pflanzen. 648. Breslau.

10. ———— '87. Sind die Wurzelanschwellungen der Erlen und Elaeagnaceen Pilzgallen? (Ber. deutsch. bot. Ges. **5**: 50).

11. Gerassimoff, J. J. '01. Über den Einfluss des Kerns auf das Wachstum der Zelle. (Bull. Soc. Imp. des Nat. de Moscou. n. s. **15**: 185–220).

12. Gravis, A. '79. Le *Schinzia alni* Woronine. (Mém. Soc. Roy. de Bot. de Belgique. **18**: 50–60).

13. Green, R. '99. The soluble ferments and fermentation. (Cambridge Natural Science Manuals).

14. Grüss, J. '93. Über den Eintritt von Diastase in das Endosperm. (Ber. deutsch. bot. Gesell. **2**: 286–292).

15. Harshberger, J. W. '03. The form and structure of the mycodomatia of *Myrica cerifera*. (Proc. Acad. Nat. Sci. of Phila. **55**: 352–361).

16. Hiltner, L. '96. Über die Bedeutung der Wurzelknöllchen von *Alnus glutinosa* für die Stickstoffernährung dieser Pflanze. (Landw. Versuchsst. **46**: 153–161).

17. ———— '99. Über die Assimilation des freien atmosphärischen Stickstoffs durch in oberirdischen Pflanzenteilen lebende Mycelien. (Centrb. für. Bakt. u. Parasitenkunde. Abt. 2. **5**: 831–837).

18. Jäger, G. '60. Über eine krankhafte Veränderung der Blüthen-Organe der Weintraube. (Flora. **43**: 49–54).

19. Kolle, W. and A. Wassermann. '03. Handbuch der pathogenen Mikroorganismen. **2**: 861–918.

20. Life, A. C. '01. The Tubercle-like rootlets of *Cycas revoluta*. (Bot. Gaz. **31**: 265–71).

21. Magnus, P. '79. Sitzungsberichte bot. Ver. Prov. Brandenburg. **21**: 119.

22. Magnus, W. '01. Studien an der endotrophen Mykorrhiza von *Neottia Nidus avis* L. (Jahrb. für wiss. Bot. **35**: 205–272).

23. Meyen, J. '29. Über das Hervorwachsen parasitischer gewäschse an den Wurzeln anderer Pflanzen. (Flora. **12**: 49–64).

24. Möller, H. '85. *Plasmodiophora alni.* (Ber. der deutsch. bot. Gesel. **3**: 102–105).

25. ———— '90. Beitrag zur Kenntniss der *Frankia subtilis* Brunchorst. (Ber. deutsch. bot. Gesel. **8**: 215–224).

26. ———— '91. Beiträge zur Kenntniss der *Frankia subtilis.* (Bot. Centrb. **45**: 60–61).

27. Nobbe, F. und L. Hiltner. '95. Vermögen auch nichtleguminosen freien Stickstoff aufzunehmen? (Landw. Versuchsst. **45**: 155–9).

28. Nobbe, F. und L. Hiltner. '99. Die endotrophe Mycorrhiza von *Podocarpus* und ihre physiologische Bedeutung. (Landw. Versuchsst. **51**: 241–5).

29. Pecklo, J. '09. Beiträge zur Lösung des Mykorrhizaproblems. (Ber. deutsch. bot. Gesel. **27**: 239–247).

30. Ratzeburg, J. T. C. '68. Die Waldverderbniss. **2**: 90, 239–242.

31. Rossmässler, E. A. '62. Der Wald. 3 ed. 460–461.

32. Schacht, H. '53. Beiträge zur Entwickelungsgeschichte der Wurzel. (Flora. **36**: 257–266).

33. ———— '53. Die Pflanzenphysiologie und Herr Dr. G. Walpers in Berlin. (Flora. **36**: 10–11).

34. ———— '54. Beiträge zur Anatomie und Physiologie der Gewächse. 160.

35. ———— '59. Lehrbuch der Anatomie und Physiologie der Gewächse. **2**: 147–148.

36. ———— '59. Grundriss der Anatomie und Physiologie der Gewächse. 121.

37. ———— '60. Der Baum. 172–174.

38. Shibata, K. '02. Cytologische Studien über die endotrophen Mykorrhizen. (Jahrb. für wiss. Bot. **37**: 643–684).

39. Sorauer, P. '85. Pflanzenkrankheiten. 2 Aufl. **1**: 747.

40. Stahl, E. '00. Der Sinn der Mykorrhizabildung. (Jahrb. f. wiss. Bot. **34**: 539–668).

41. Ternetz, Charlotte. '04. Assimilation des atmosphärischen Stickstoffs durch einen torfbewohnenden Pilz. (Ber. deutsch. bot. Ges. **22**: 267).

42. Von Tubeuf, K. '95. Pflanzenkrankheiten durch kryptogame Parasiten verursacht. 117. Berlin.—Mykodomatien der Erlen, Elaeagnaceen und Myricaceen, veranlasst durch *Frankia*-Arten.

43. Van Tiegham, Ph. '79. Sur la fermentation de la cellulose. (Bull. Soc. Bot. de France **26**: 25–31).

44. Vines, S. H. '97. Proteolytic enzymes of *Nepenthes*. (Annals of Botany. **11**: 563–584).

45. ———— '02. Tryptophane in proteolysis. (Annals of Botany. **16**: 1–22).

46. ———— '03. Proteolytic enzymes in plants—I. (Annals of Botany. **17**: 237–264).

47. ———— '03. Proteolytic enzymes—II. (Annals of Botany. **17**: 597–616).

48. ———— '04. Proteases of plants. (Annals of Botany. **18**: 289–317).

49. ———— '09. Proteases of plants. (Annals of Botany. **23**: 1–18).

50. ———— '10. Proteases of plants. (Annals of Botany. **24**: 213–222).

51. Ward, H. Marshall. '88. A lily disease. (Annals of Botany. **2**: 319–382).

52. Warming, E. '76. Smaa biologiske og morfologiske Bidrag. (Botanisk Tidsskrift. iii. **1**: 84–110).

53. Wilson, Robt. W. '09. Research on proteolytic enzymes in fungi and bacteria, (Notes fr. Roy. Bot. Gard. Edin. **21**: 27–37).

54. Wolpert, J. '09. Die Mycorrhizen von *Alnus*. (Flora. **100**: 60–67).

55. Woodhead, F. W. '00. On the structure of the root nodules of *Alnus glutinosa*. (Rep. British Assoc. Adv. Sci. **1900**: 931).

56. Woronin, M. '66. Über die bei der Schwarzerle (*Alnus glutinosa*) und der gewöhnlichen Garten-Lupine auftretenden Wurzelanschwellungen. (Mém. Acad. Imp. de St. Petersbourg. vii. **10**^{6}: 1–13).

57. ———— '85. Bemerkung zu dem Aufsatze von Herrn H. Möller über *Plasmodiophora alni*. (Ber. deutsch. bot. Gesel. **3**: 177–178).

58. Zach, F. '08. Über den in den Wurzelknöllchen von *Elaeagnus angustifolia* und *Alnus glutinosa* lebenden Fadenpilz. (Sitz. d. kais. Ak. Wiss. zu. Wien. Math. u. Naturw. Kl. **117**: 973–983).

59. ———— '09. Untersuchungen über die Kurzwurzel von *Sempervivum* und die daselbst auftretende endotrophe Mykorrhiza. (Sitzber. der kais. Akad. d. Wiss. zu Wien. Mäth. u. Natur. Kl. **118**: 185–200).

60. ———— '10. Studie über Phagocytose in den Wurzelknöllchen der Cycadeen. (Bot. Zeitschrift. **60**: 49–55).

EXPLANATION OF PLATES.

All of the figures were drawn with the aid of a camera lucida. A Leitz 1/12 oil immersion objective and ocular no. 4, giving a magnification of 1350 diameters, were used for all except figures 1, 14, and 29, for which use was made of a no. 3 objective and no. 1 ocular, giving a magnification of 85 diameters.

Plate 6.—A portion of a *Ceanothus* root with many young tubercles on the small lateral roots, above. Part of a *Ceanothus* root on which older tubercles are formed into loose clusters, below.

Plate 7.—A large *Elaeagnus* root showing the dense mass of tubercles attached to it by a short branch, above. A portion of a root with large clusters, below.

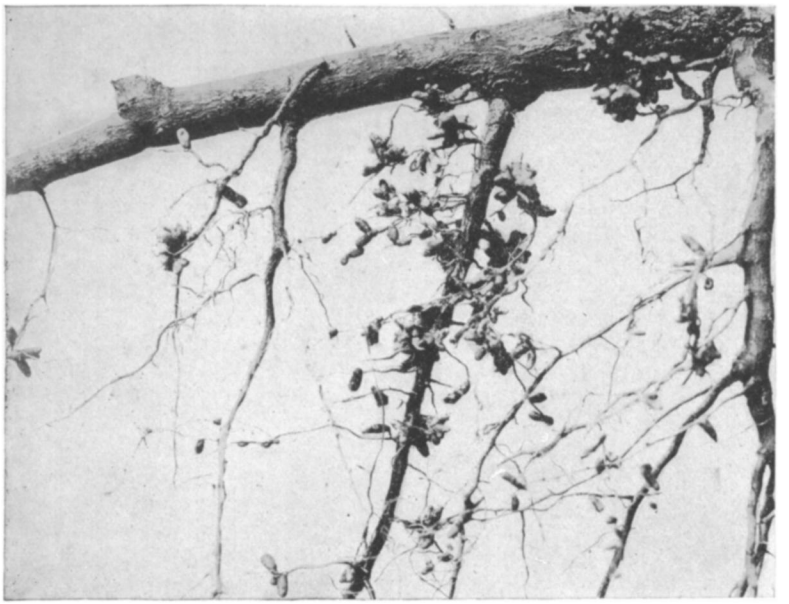
Plate 8.—Part of a stem of *Myrica cerifera* showing the masses formed at the ends of short adventitious roots.

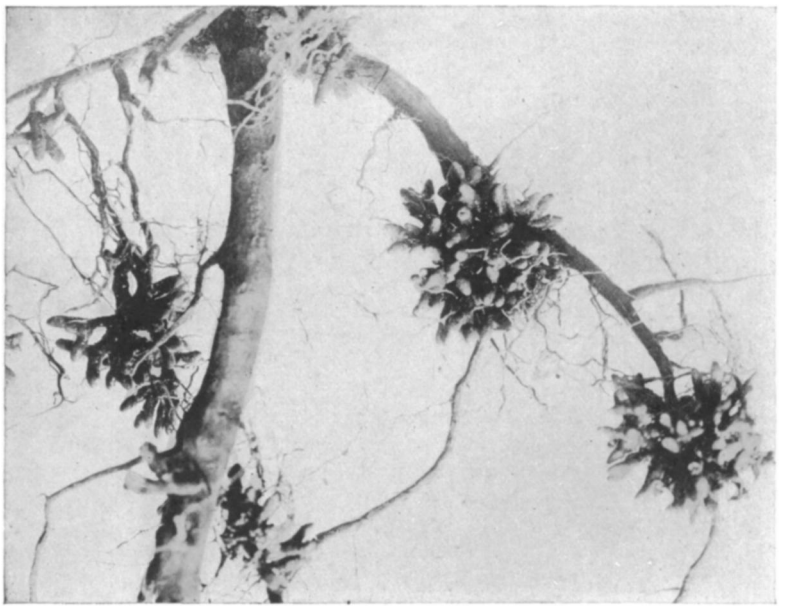
Plates 9–10.—*Ceanothus americanus.* 1, Transverse section of a root tubercle of *Ceanothus* indicating the infected region and some of the fungal stages. 2, Cells of the meristematic region showing early stages of infection. One cell shows where a hypha is just entering. The cell wall between some of the cells is being dissolved. 3, An embryonic cell just infected showing the branched mycelium. 4, An older hypertrophied host cell with enlarged nucleus. The mycelium is much branched and entwined. No vesicles have yet been formed; portions of the cell walls are being dissolved. 5, 6, Nuclei of the fungus set forth by the haematoxylin stain. The hyphal walls are difficult to differentiate. 7, A stage where the sporanges are formed at the end of the hyphal branches. Infection of adjoining cells is also shown. 8, Hypertrophied nuclei of the host cell differing from the following. 9, Nuclei of digestive cells similar to the one shown in f. 7. 10, Young sporanges showing their content. 11, Older and mature vesicles with a single nucleus. 12, Sporanges burst open, the content has disappeared. 13, A cell showing the last stage of the fungus where all but the walls of the mycelium is absorbed.

Plates 10–12.—*Elaeagnus argentea.* 14, Cross section showing the infected region of the tubercle and its various tissues. The large dense cells contain the fungus in the vesicular stage,—other cells show younger stages. 15, Two uninfected cells showing nucleus, large starch grains and fine granular cytoplasm. 16, An infected cell showing hypertrophied nucleus, the mass of mycelial threads and the mode of infection. 17, 18, Infected cells where the hyphae pass through the cell wall into adjoining cells. 19, A large hypertrophied cell with an amoeboid nucleus. The fungus has the sporanges, in

which the content is broken up into parts. 20, 21, The same vesicles drawn on a larger scale. 22, A stage where the fungus is partially destroyed, the walls of the vesicle and hyphae remaining. 23, A host cell which has collapsed in which the remains of the fungus are still present. 24, Nuclei of the host cell before they become amoeboid in form. 25, Stages of nuclei found in the digestive cells. 26, Very late stages of degeneration of nuclei just before their final disappearance.

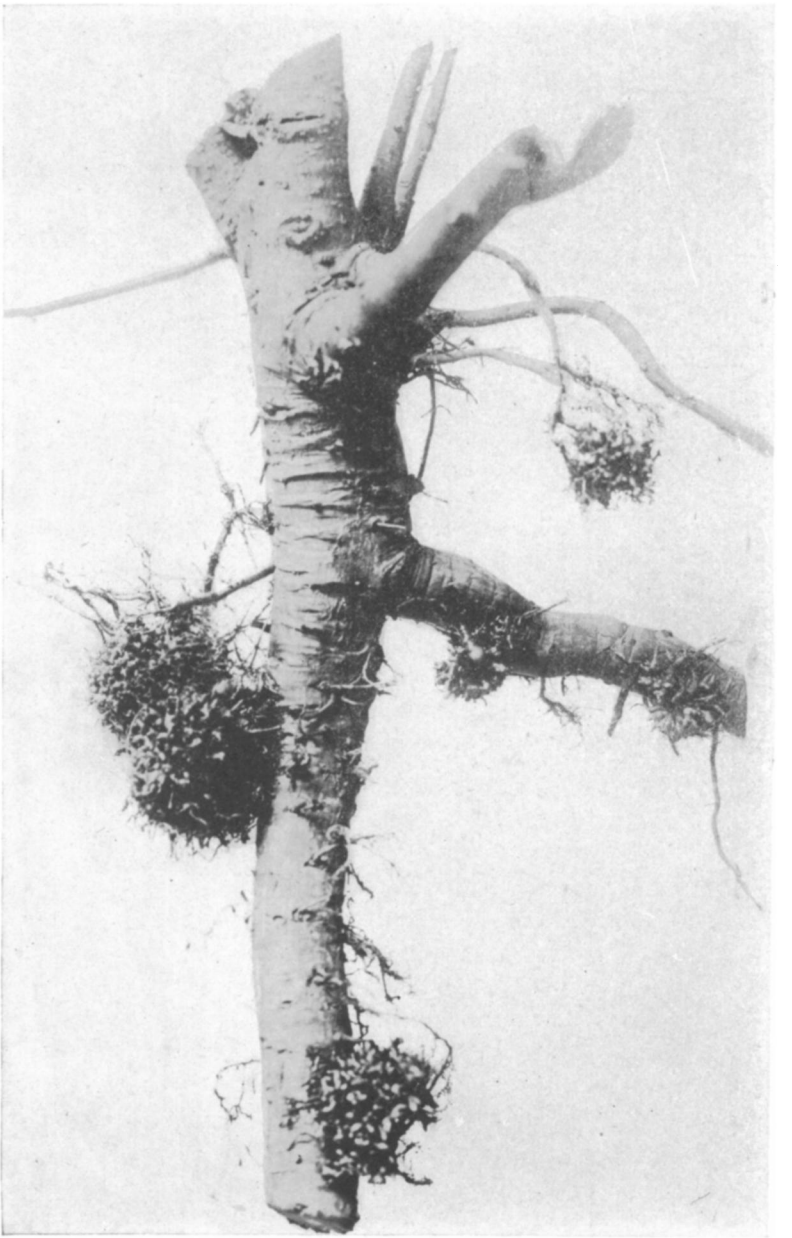
Plates 13, 14.—*Myrica cerifera.* 28, Life-sized tubercles as they are found in the clusters. 29, A longitudinal section of a tubercle showing the infected region and the various tissues of the tubercle. 30, Several uninfected cells showing the cell content. 31, Cells indicating the method by which internal infection takes place. 32, Cells showing the large number of hyphae which pass through the walls to infect the cell. 33, Several cells of the infected region showing young and old stages of the fungus where the branches of the hyphae have enlarged into club-shaped structures. 34, A portion of mycelial thread showing the nuclei. 35, The club-shaped ends of the hyphae. 36, The same but older structures where the nuclei have passed into them from the mycelium. 37, A stage where the host cell and the nucleus begin to disintegrate. The fungus also shows similar stages. 38, Several degenerating nuclei found in host cells.



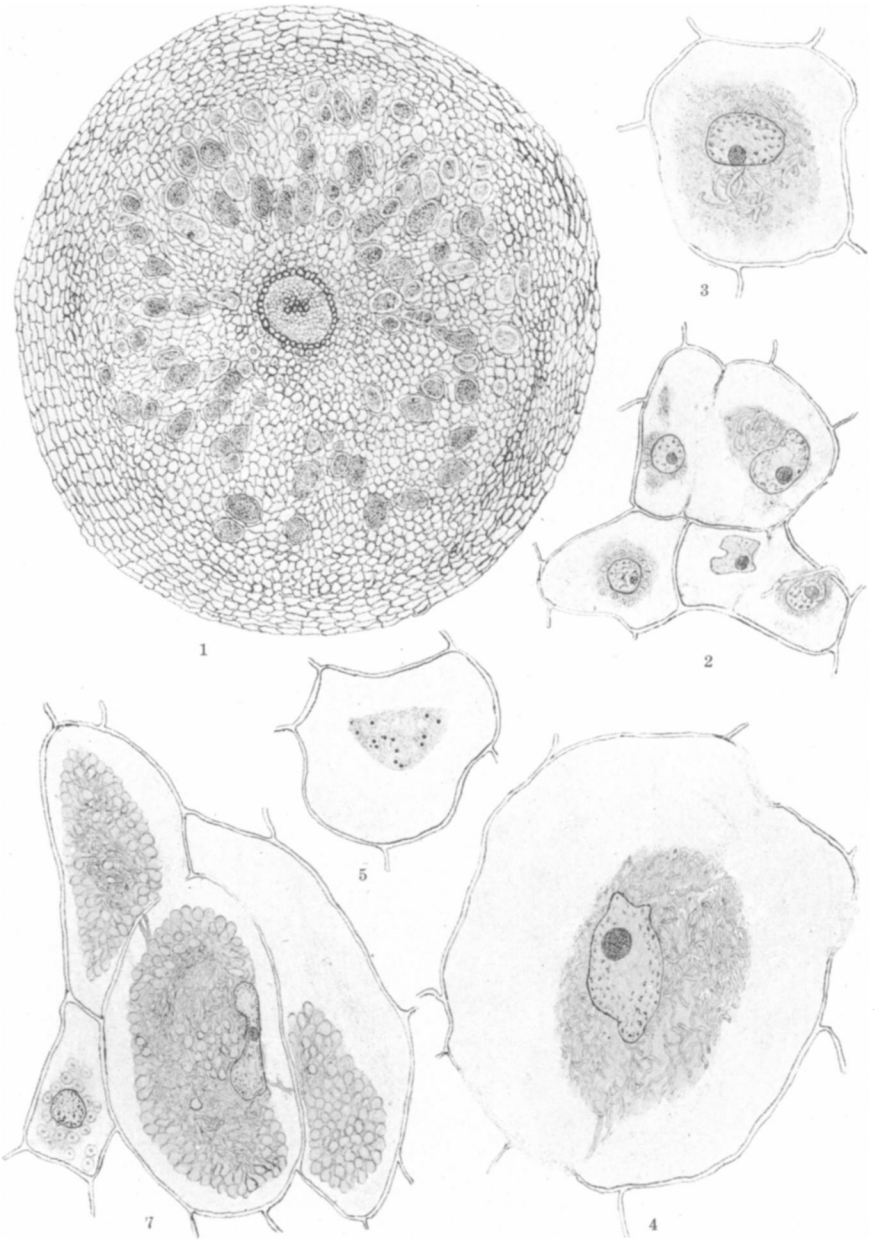


CEANOTHUS.

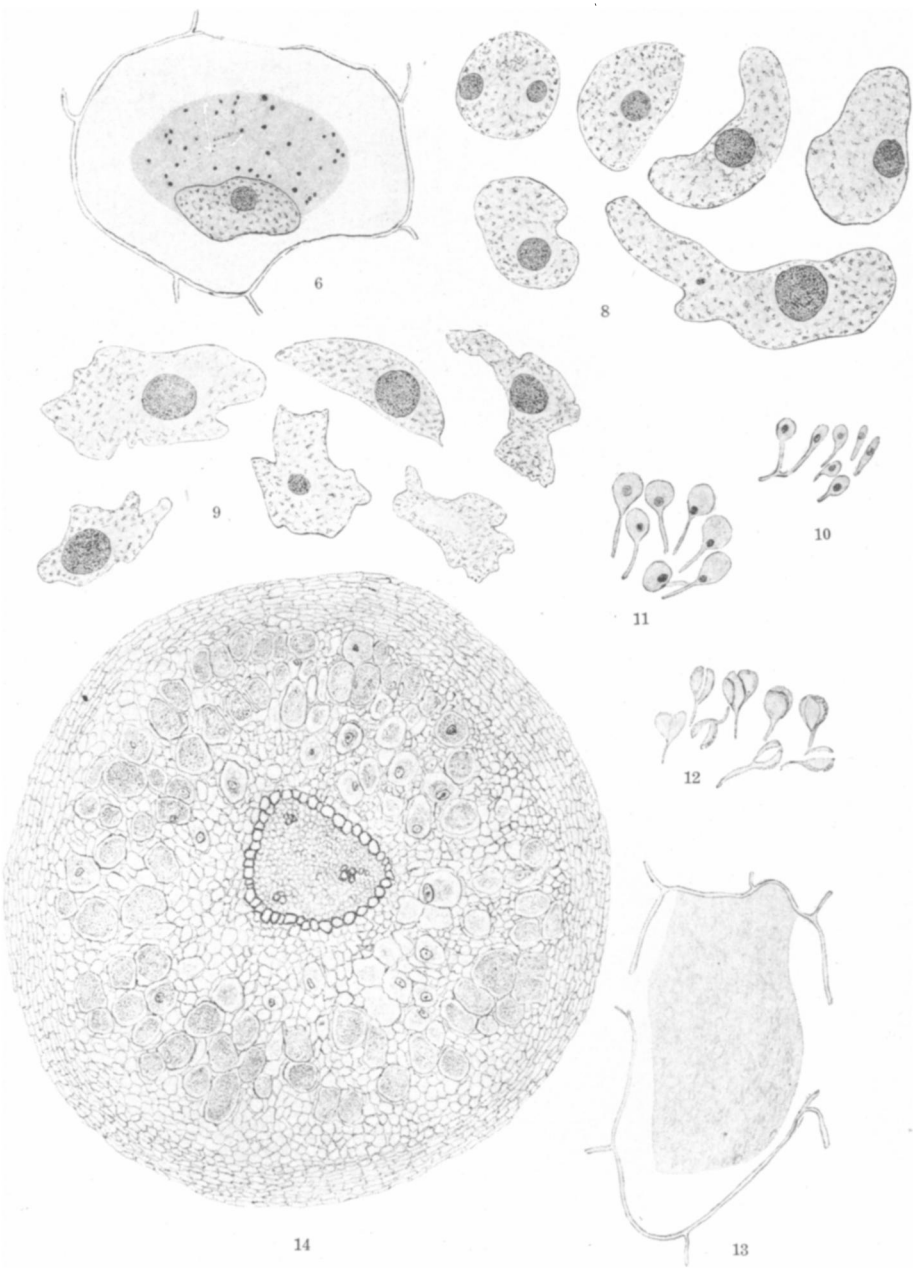
ELAEAGNUS.



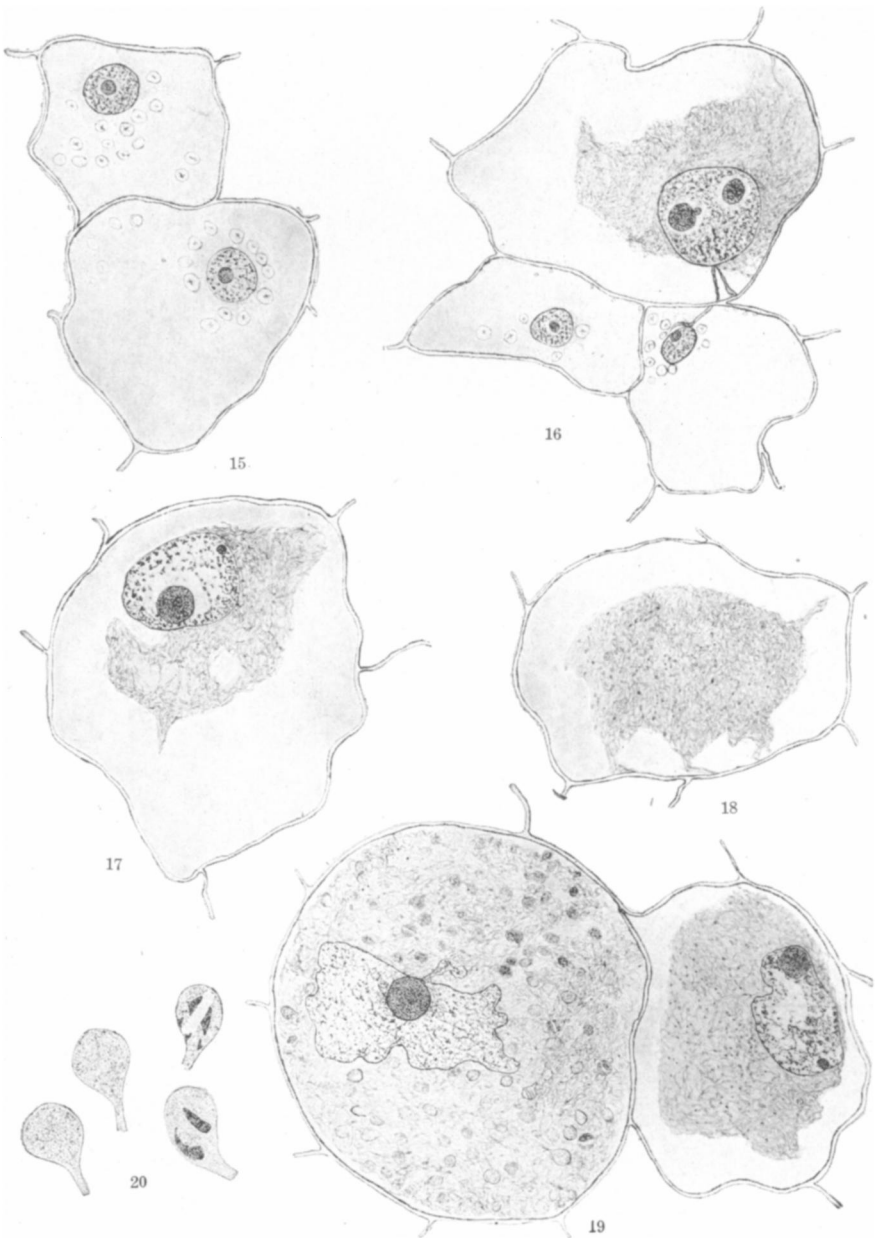
MYRICA.



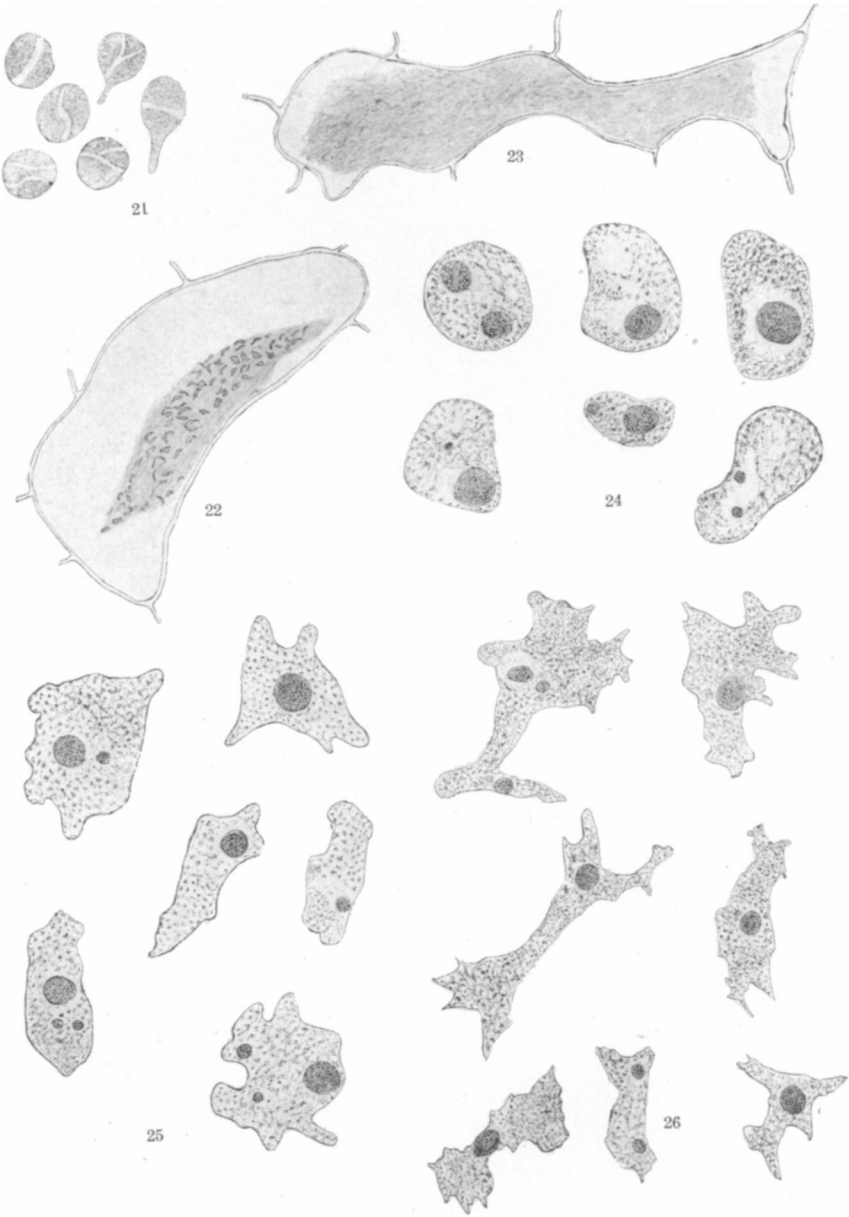
CEANOTHUS.



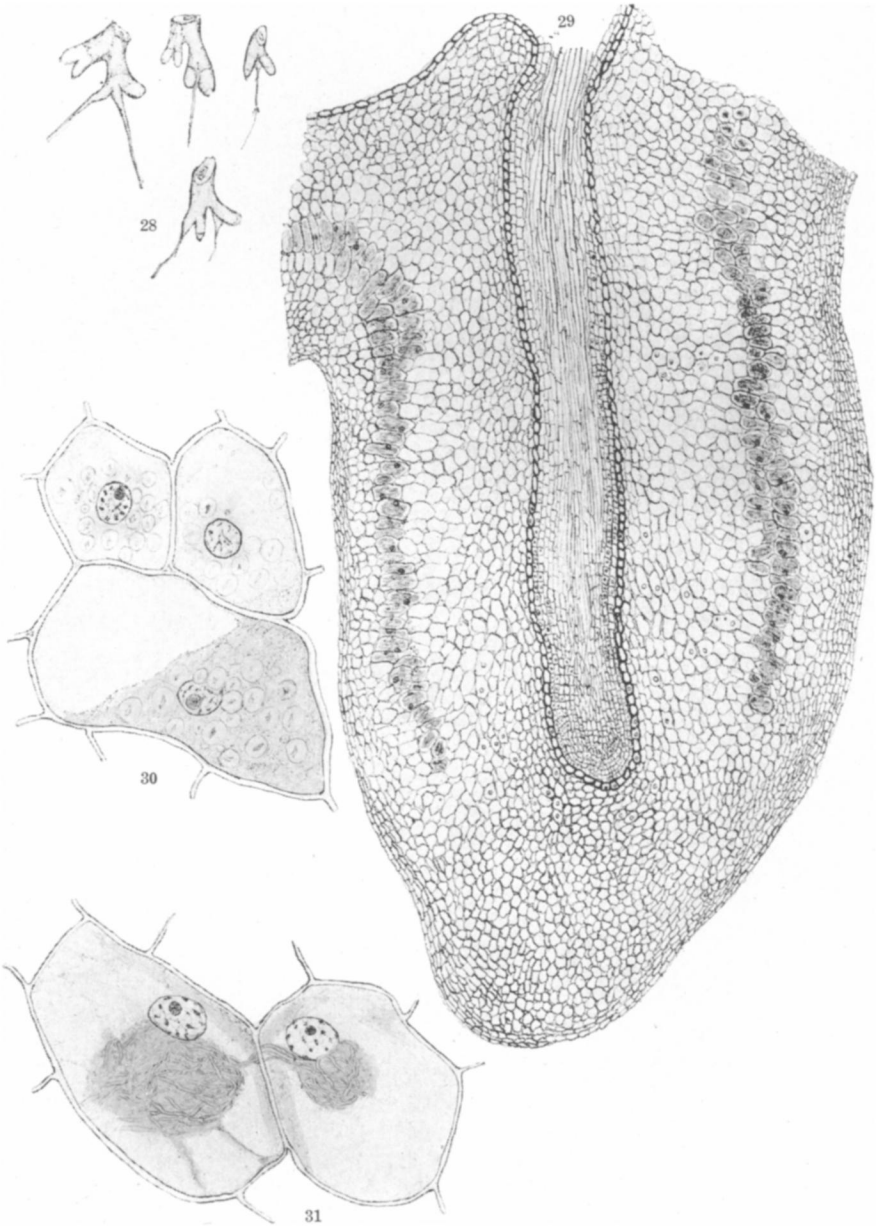
CEANOTHUS AND ELAEAGNUS.



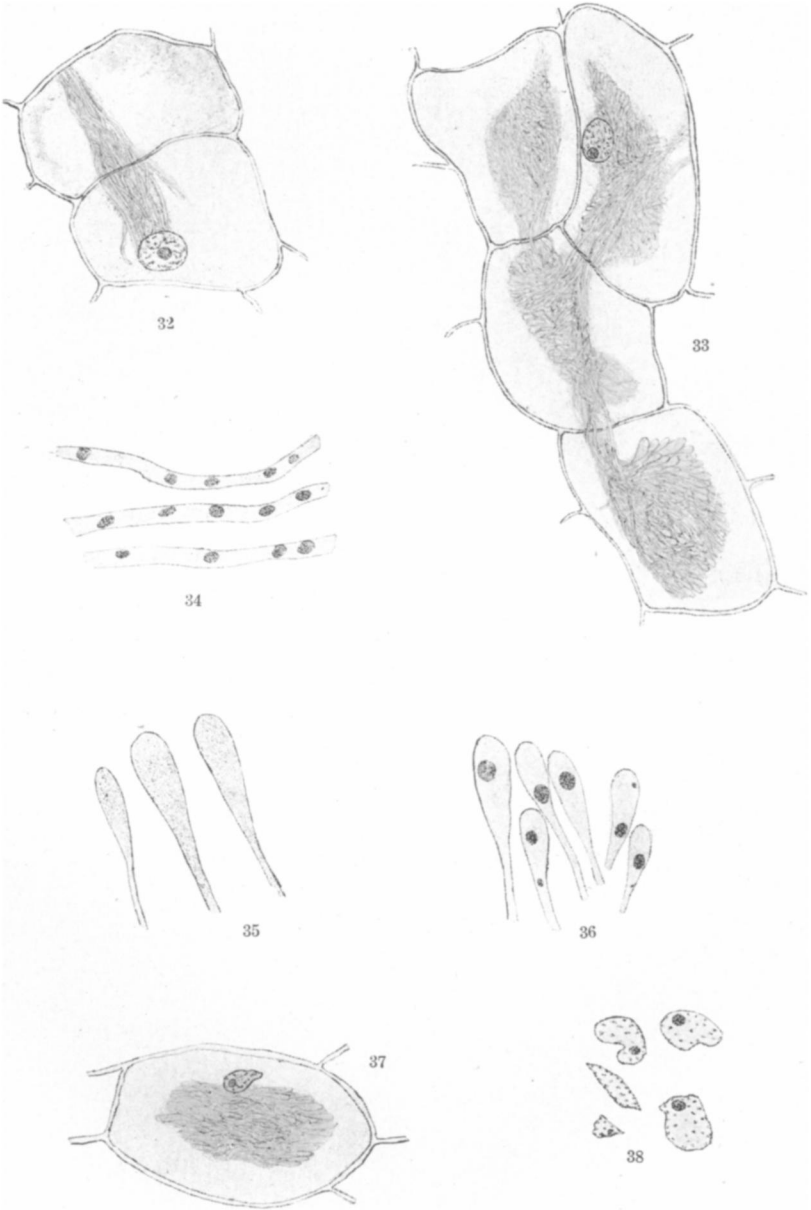
ELAEAGNUS.



ELAEAGNUS.



MYRICA.



MYRICA.